

# BD LSR II

## User's Guide

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**BD Biosciences**  
2350 Qume Drive  
San Jose, CA 95131-1807  
USA  
Tel (877) 232-8995  
Fax (408) 954-2347

**Asia Pacific**  
Tel (65) 6-861-0633  
Fax (65) 6-860-1590

**Europe**  
Tel (32) 53-720211  
Fax (32) 53-720450

**Brazil**  
Tel (55) 11-5185-9995  
Fax (55) 11-5185-9895

**Japan**  
Nippon Becton Dickinson Company, Ltd.  
Tel 0120-8555-90

**Canada**  
Tel (888) 259-0187  
(905) 542-8028  
Fax (905) 542-9391  
[canada@bd.com](mailto:canada@bd.com)

**Mexico**  
Tel (52) 55-5999-8296  
Fax (52) 55-5999-8288

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Guide revised by Denise Lee and Lisa Gibson; edited and produced by Pushpa MacFarlane.

## **Patents**

BD LSR II is covered by one or more of the following US patents and foreign equivalents: 4,745,285; 4,844,610; and 6,014,904.

PE and APC: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942

PerCP: US 4,876,190

Cy5.5 and Cy7: US 5,268,486; 5,486,616; 5,569,587; 5,569,766; and 5,627,027

Pe-Cy7: US 4,542,104

APC-Cy7: US 5,714,386

BD FACS Lysing Solution: US 4,654,312; 4,902,613; and 5,098,849

## Regulatory Information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

**WARNING:** Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

**NOTE:** This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, can cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense.

Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.

## History

Revision	Date	Change Made
334717 Rev. A	12/02	Initial release
334717 Rev. B	2/03	Second release



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# About This Guide

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This user's guide contains the instructions necessary to operate and maintain your BD™ LSR II flow cytometer. Because many instrument functions are controlled by BD FACSDiVa™ software, this guide also contains basic software information needed for instrument setup. For more detailed instructions on how to use the software, refer to the *BD FACSDiVa Software User's Guide*.

The *BD LSR II User's Guide* assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, refer to the documentation provided with your computer.

The *BD LSR II User's Guide* contains the following chapters:

- Chapter 1 describes BD LSR II system components.
- Chapter 2 and Chapter 3 detail instrument-specific software components, setting up the BD LSR II flow cytometer, and running samples.
- Chapter 4 and Chapter 5 provide application-specific instructions for DNA analysis and calcium flux.
- Chapter 6 describes maintenance procedures.
- Chapter 7 details instrument troubleshooting.

You can find a technical overview of BD LSR II flow cytometer fluidics, optics in Appendix A, a sample quality control (QC) log in Appendix B, and empty filter configuration templates in Appendix C.

# Conventions

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The following tables list conventions used throughout this guide.

Table 1 lists the symbols that are used to alert you to a potential hazard. The word accompanying the hazard symbol indicates the level of severity, as shown in Table 2.

**Table 1** Hazard icons

Symbol	Hazard	Symbol	Hazard
	Electrical		General or ergonomic
	Laser		
	Biological		

**Table 2** Severity indicators<sup>a</sup>

Indicator	Level of Severity
<b>WARNING</b>	The hazard or unsafe practice could result in severe injury or death.
<b>CAUTION</b>	The hazard or unsafe practice could result in minor injury.
<b>NOTE</b>	The hazard or unsafe practice could result in a possibly dangerous situation; material could be damaged.

a. In compliance with the ANSI Z535.4 standard: Product Safety Signs and Labels

Table 3 lists another helpful icon used in the guide, and Table 4 lists text and keyboard conventions.

**Table 3** Notice icons

Icon	Notice Type	Use
	<b>Tip</b>	Highlights features or hints that can save time and prevent difficulties

**Table 4** Text and keyboard conventions

Convention	Use
<i>Italics</i>	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, “choose File > Print” means to choose Print from the File menu.
Ctrl-X	When used with key names, a dash means to press two keys simultaneously. For example, Ctrl-P means to hold down the Control key while pressing the letter <i>p</i> .

# Technical Assistance

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For technical questions or assistance in solving a problem, read the section of the user's guide specific to the operation you are performing.

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.

When contacting BD Biosciences, have the following information available:

- product name, part number, and serial number
- any error messages
- details of recent system performance

For instrument support from within the US, call (877) 232-8995, prompt #2-2.

US address:

2350 Qume Drive  
San Jose, CA 95131-1807

For support from within Canada, call (888) 259-0187.

Customers outside the US and Canada, contact your local BD representative or distributor.

# Safety and Limitations

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The BD LSR II flow cytometer is equipped with safety features for your protection. Operate the instrument only as directed in this user's guide and the *BD LSR II Safety and Limitations* booklet. Keep this information available for future reference and for new users.

## Laser Safety

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Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. The main hazard of laser radiation is direct or indirect exposure of the eye to thermal radiation from the visible and near-infrared spectral regions (325–1400 nm). Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness.

There are other potentially serious hazards in other spectral regions. Excessive ultraviolet exposure produces an intolerance to light (photophobia) accompanied by redness, a tearing discharge from the mucous membrane lining the inner surface of the eyelid (conjunctiva), shedding of the corneal cell layer surface (exfoliation), and stromal haze. These symptoms are associated with photokeratitis, otherwise known as snow blindness or welder's flash, which results from radiant energy-induced damage to the outer epidermal cell layer of the cornea. These effects can be the result of laser exposure lasting only a fraction of a second.

# Laser Classification

Laser hazard levels depend on laser energy content and the wavelengths used. Therefore, it is impossible to apply common safety measures to all lasers. A numbered system is used to categorize lasers according to different hazard levels. The higher the classification number, the greater the potential hazard. The BD LSR II flow cytometer is a Class I laser product per 21 CFR Subchapter J and Class 1 laser product per EN 60825-1:1994+ A2:2001. The lasers are fully contained within the instrument structure and call for no special work area safety requirements except during service procedures. These procedures are to be carried out only by BD Biosciences service personnel.

We recommend that you operate your BD LSR II flow cytometer only with the optics cover in place and the instrument lids closed. When operated under these conditions, the instrument poses no danger of exposure to hazardous laser radiation.

 **WARNING** To prevent injury, do not modify the optics cover and laser shielding in any way.

 **WARNING** Only BD Biosciences service personnel should perform service procedures on the laser equipment and the BD LSR II instrument.

## Precautions for Safe Operation

To reduce the possibility of hazardous exposure to radiant laser energy, do not remove caution labels from the instrument. Caution labels are located on all removable covers or any place where the laser beam can emerge from the instrument.

 **WARNING** Controls, adjustments, or performance of procedures other than those specified in the *BD LSR II User's Guide* can result in exposure to hazardous visible or ultraviolet laser radiation, or both.

# Electrical Safety

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 **WARNING** Lethal electrical hazards can be present in all lasers, particularly in laser power supplies. Every portion of the electrical system, including the printed circuit boards, should be considered to be at a dangerous voltage level. Avoid potential shock by following these guidelines.

- Connect the equipment only to an approved power source. Do not use extension cords. Have an electrician immediately replace any damaged cords, plugs, or cables. Refer to the *BD LSR II Facilities Requirement Guide* for specific information.
- Do not remove the grounding prong from the power plug. Have a qualified electrician replace any ungrounded receptacles with properly grounded receptacles in accordance with the local electrical code.
- For installation outside the US, use a power transformer or conditioner to convert the local power source to meet the BD LSR II power requirements (120 V  $\pm$ 10%, 50/60 Hz). Contact your local BD office for further information.

# General Safety

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 **WARNING** The instrument handles are for BD Biosciences authorized personnel only. Do not access them or attempt to lift the instrument with them, or you could injure yourself.

 **CAUTION** To avoid burns, do not touch the fan guards on the back of the instrument. The fan guards could be hot during and after instrument operation.

# Biological Safety

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 **WARNING** All biological specimens and materials coming into contact with them are considered biohazardous. Avoid exposure to biohazardous material by following these guidelines.

- Handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.
- Expose waste container contents to bleach (10% of total volume) for 30 minutes before disposal. Dispose of waste in accordance with local regulations. Use proper precaution and wear suitable protective clothing, eyewear, and gloves.
- Prevent waste overflow by emptying the waste container frequently or whenever the waste management system alarms.

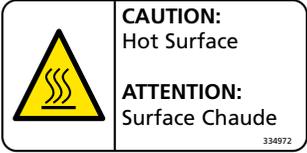
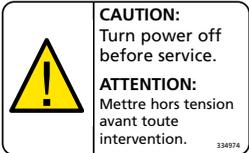
For information on laboratory safety, refer to the following guidelines. NCCLS documents can be ordered online at [www.nccls.org](http://www.nccls.org).

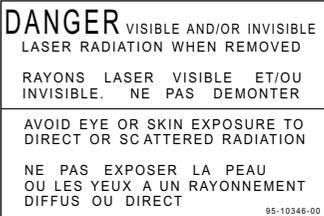
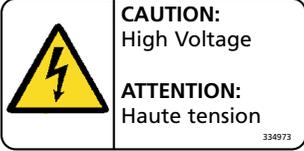
- *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline.* Wayne, PA: National Committee for Clinical Laboratory Standards, 1997. NCCLS document M29-A.
- *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline.* Wayne, PA: National Committee for Clinical Laboratory Standards; 1990. NCCLS document H18-A.

# Symbols and Labels

The following symbols, warnings, or precaution labels appear on the BD LSR II flow cytometer or the waste and fluid tanks.

Symbol	Meaning	Location(s)
	Dangerous voltage	Rear instrument panel near power receptacle
	Laser radiation hazard	Near all removable covers and any place where the laser beam can emerge from the instrument
	Caution! Consult accompanying documents.	Near the instrument handles

Label	Meaning	Location(s)
	Caution Hot surface	Rear instrument panel adjacent to exhaust fans
	CAUTION Turn power off before service.	<ul style="list-style-type: none"> <li>• Rear instrument panel adjacent to power receptacle</li> <li>• Near internal power shield</li> </ul>

Label	Meaning	Location(s)
 <p><b>DANGER</b> VISIBLE AND/OR INVISIBLE LASER RADIATION WHEN REMOVED RAYONS LASER VISIBLE ET/OU INVISIBLE. NE PAS DEMONTER</p> <p>AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION NE PAS EXPOSER LA PEAU OU LES YEUX A UN RAYONNEMENT DIFFUS OU DIRECT</p> <p>95-10346-00</p>	<p>DANGER</p> <p>Visible and/or invisible laser radiation when removed. Avoid eye or skin exposure to direct or scattered radiation.</p>	<ul style="list-style-type: none"> <li>• Optics cover</li> <li>• Lasers</li> </ul>
 <p><b>CAUTION:</b> High Voltage</p> <p><b>ATTENTION:</b> Haute tension</p> <p>334973</p>	<p>CAUTION</p> <p>High voltage</p>	<p>Rear instrument panel adjacent to power receptacle</p>
 <p><b>WASTE:</b> CAUTION: Handle biological substances with care. Refer to manual for handling procedure.</p> <p><b>DECHETS:</b> ATTENTION: Manipuler les substances biologiques avec precaution. Se reporter au manual pour les consignes a suivre.</p> <p>334971</p>	<p>WASTE</p> <p>Caution: Handle biological substances with care. Refer to manual for handling procedure.</p>	<p>System waste tank</p>
 <p><b>DANGER</b> VISIBLE AND/OR INVISIBLE LASER RADIATION WHEN REMOVED. AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION.</p>	<p>DANGER</p> <p>Visible and/or invisible laser radiation when removed. Avoid eye or skin exposure to direct or scattered radiation.</p>	<p>Near all removable covers and any place where the laser beam can emerge from the instrument</p>
 <p><b>DANGER</b> RAYONNEMENT LASER VISIBLE ET/OU INVISIBLE LORS DU RETRAIT EVITER L'EXPOSITION OCULAIRE OU CUTANEE AU RAYONNEMENT DIRECT OU DIFFUSE</p>		

# Limitations

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This instrument is for Research Use Only. Not for use in diagnostic or therapeutic procedures.



# 1

## Introduction

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The following topics are covered in this chapter:

- Overview on page 24
- Fluidics on page 25
- Optics on page 30
- Electronics on page 35

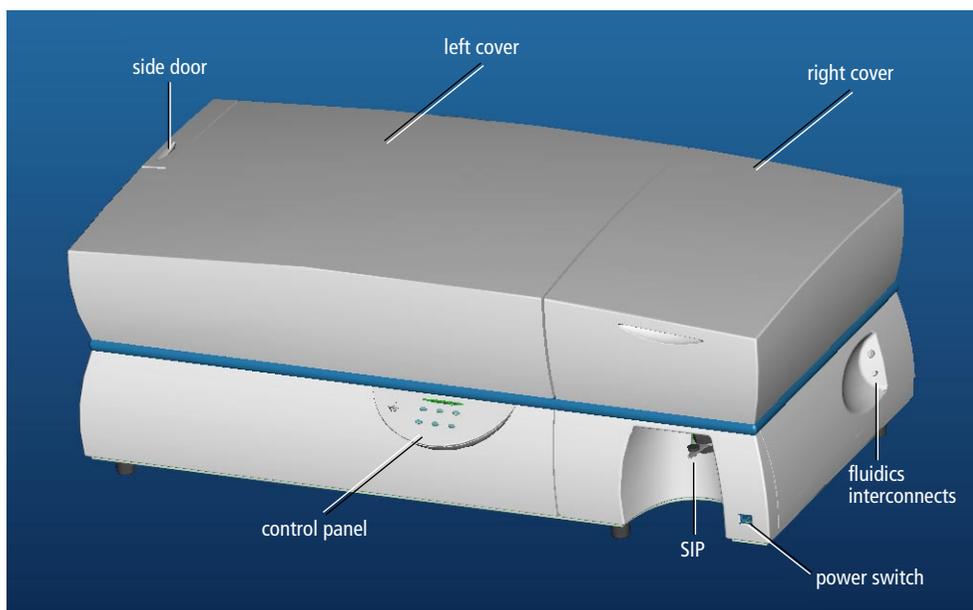
# Overview

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The BD LSR II is an air-cooled four-laser benchtop flow cytometer with the ability to acquire up to 10 colors. It uses fixed-alignment lasers that transmit light via mirrors through a flow cell to user-configurable Octagon and Trigon detector arrays. These detectors collect and translate fluorescence signals into electronic signals. Instrument electronics then convert the signals into digital data.

# Components

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**Figure 1-1** BD LSR II flow cytometer

## Power Switch

The power switch is located on the lower-right side of the BD LSR II instrument as shown in Figure 1-1.

## Control Panel

The control panel contains fluidics controls (Figure 1-2). For a description of the controls, see the following sections.

Fluidics controls	page 26
Sample flow rate control	page 26

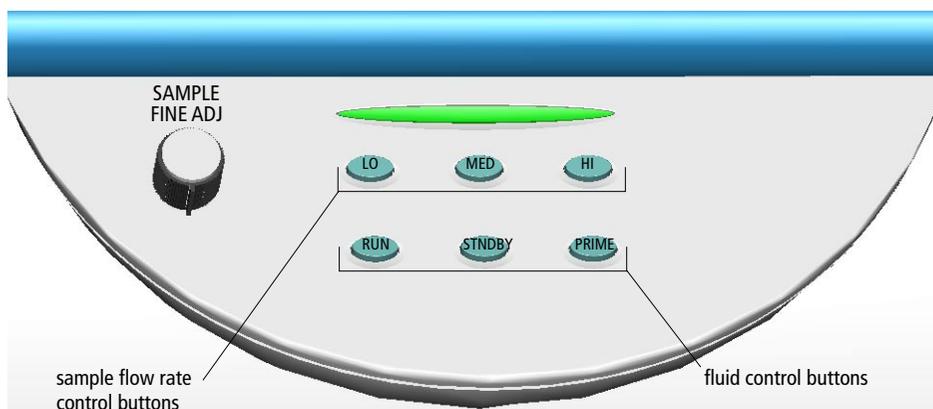


Figure 1-2 Control panel

## Handles

**⚠ WARNING** The instrument handles are for BD Biosciences authorized personnel only. Do not access them or attempt to lift the instrument with them, or you could injure yourself.

## Fluidics

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The purpose of the fluidics system is to carry the sample out of the sample tube and into the sensing region of the flow cell. Cells are carried in the sample core stream in single file and measured individually.

## Sample Flow Rate Control

Three flow rate control buttons—LO, MED, and HI—set the sample flow rate through the flow cell. The Sample Fine Adjust knob allows you to adjust the rate to intermediate levels.

When the sample fine adjust knob is at its midpoint, the sample flow rates at the LO, MED, and HI settings are approximately 12, 35, and 60  $\mu\text{L}/\text{min}$  of sample, respectively. The knob turns five full revolutions in either direction from its midpoint, providing sample flow rates from 0.5–2X the midpoint value. For example, if the LO button is pressed, the knob will give flow rates from approximately 6–24  $\mu\text{L}/\text{min}$ .

## Fluid Control

Three fluid control buttons—RUN, STNDBY, and PRIME—set the instrument mode (Figure 1-2 on page 25).

- RUN pressurizes the sample tube to transport the sample through the sample injection tube and into the flow cell.

The RUN button is green when the sample tube is on and the support arm is centered. When the tube support arm is moved left or right to remove a sample tube, the instrument switches to an automatic standby status to conserve sheath fluid—the RUN button changes to orange.

- STNDBY (standby) stops fluid flow to conserve sheath fluid.

If leaving the instrument for more than a few minutes, place a tube containing 1 mL of deionized (DI) water on the sample injection port (SIP) and press STNDBY.

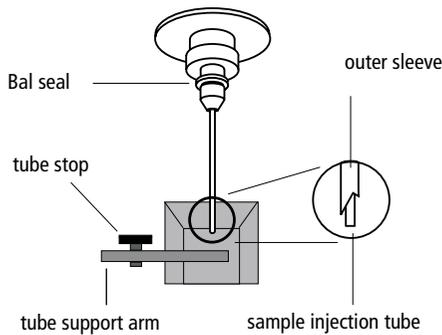
- PRIME prepares the fluidics system by draining and filling the flow cell with sheath fluid.

The fluid flow initially stops and pressure is reversed to force fluid out of the flow cell and into the waste container. After a preset time, the flow cell

fills with sheath fluid at a controlled rate to prevent bubble formation or entrapment. At completion, the instrument switches to STNDBY mode.

## Sample Injection Port

The sample injection port (SIP) is where the sample tube is installed. The SIP includes the sample injection tube and the tube support arm. Samples are introduced through a stainless steel injection tube equipped with an outer droplet containment sleeve. The sleeve works in conjunction with a vacuum pump to eliminate droplet formation of sheath fluid as it backflushes from the sample injection tube.



**Figure 1-3** Sample injection port (SIP)

- Sample injection tube—stainless steel tube that carries sample from the sample tube to the flow cell. This tube is covered with an outer sleeve that serves as part of the droplet containment system.
- Tube support arm—arm that supports the sample tube and activates the droplet containment system vacuum. The vacuum is on when the arm is positioned to the side and off when the arm is centered.
- Droplet containment system—prevents sheath fluid from dripping from the SIP and provides biohazard protection

When no sample tube is installed on the SIP, sheath fluid backflushes through the sample injection tube. This backflush helps prevent carryover

of cells between samples. The droplet containment system vacuum is activated when the sample tube is removed and the tube support arm is moved to the side. Sheath fluid is aspirated as it backflushes the sample injection tube.

 **NOTE** If a sample tube is left on the SIP with the tube support arm to the side (vacuum on), sample will be aspirated into the waste container.

## Sheath and Waste Containers

The sheath and waste containers are outside the instrument and can be stored on the floor.

### Sheath Container

The sheath container has a capacity of 8 L. Sheath fluid is filtered through an in-line, interchangeable filter that prevents small particles from entering the sheath fluid lines. The sheath filter is located on top of the sheath container.

Before opening the sheath container:

- 1** Put the instrument in STNDBY mode.
- 2** Disconnect the air line (green).
- 3** Depressurize the sheath container by lifting its vent cap.

### Waste Container

The waste container has a capacity of 20 L. An alarm sounds when the container becomes full.

 **WARNING** To avoid leakage of biohazardous waste, put the instrument in STNDBY mode before disconnecting the waste container.

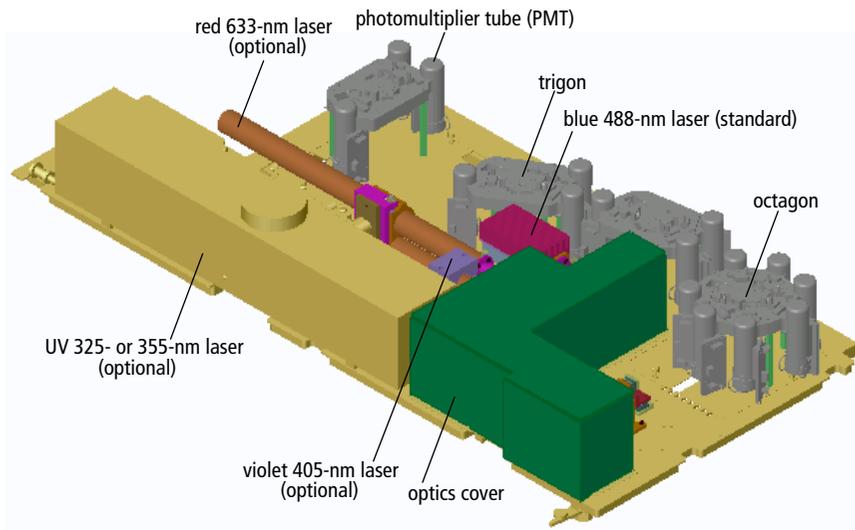
 **WARNING** The waste container contents might be biohazardous. Treat contents with bleach (10% of total volume). Dispose of waste with proper precautions in

accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

 **CAUTION** The waste container is heavy when full. When emptying it, use good body mechanics to prevent injury.

# Optics

Figure 1-4 shows the optical bench components of the BD LSR II instrument.



**Figure 1-4** Optical bench components

photomultiplier tubes (PMTs)	Devices that convert optical signals into electrical signals (See Detectors on page 33.)
Octagon	Array of PMTs and filters that can detect side scatter (SSC) and up to four fluorescence signals (See Figure 1-5 on page 33.)
Trigon	Array of PMTs and filters that can detect up to two fluorescence signals (See Figure 1-6 on page 34.)
lasers: blue 488- , red 633- , UV 325- or 355- , and violet 405-nm	Information about lasers on page 31
optics cover	Shielding that houses the flow cell, forward scatter (FSC) assembly (see Detectors on page 33), and the excitation optics

## Lasers

The BD LSR II flow cytometer has a fixed-alignment, 488-nm laser with the option of three additional alignment-free lasers.

**Table 1-1** BD LSR II flow cytometer laser options

	Laser	Type	Wavelength (Color)	Power (mW)	Warm-Up Time (min)
Standard	Coherent Sapphire™	solid state	488 nm (blue)	20	30
	• Kimmon HeCd	Helium-Cadmium gas	325 nm (UV)	8	60
Optional	or				
	• Lightwave Xcite™	solid state	355 nm (UV)	20	30
	Coherent Vioflame™	solid state	405 nm (violet)	25	15
	JDS Uniphase 1344P	Helium-Neon (HeNe) gas	633 nm (red)	17	20

- The primary blue 488-nm laser (Coherent Sapphire) generates forward scatter (FSC) and side scatter (SSC) signals and up to four fluorescence signals.
- The optional ultraviolet laser (Kimmon HeCd 325 nm or Lightwave Xcite solid state 355 nm) generates two additional fluorescence signals.
- The optional violet 405-nm laser (Coherent VioFlame) generates two fluorescence signals.
- The optional red 633-nm laser (JDS Uniphase HeNe) generates two fluorescence signals.

 **NOTE** To extend the life of the UV 325-nm HeCd laser, turn on the instrument for at least four hours every week.

# Filters

Optical filters attenuate light or help direct it to the appropriate detectors. The BD LSR II instrument uses dichroic filters. Dichroic filters transmit light of a specific wavelength, while reflecting other wavelengths. The name and spectral characteristics of each filter appear on its holder.

There are three types of dichroic filters:

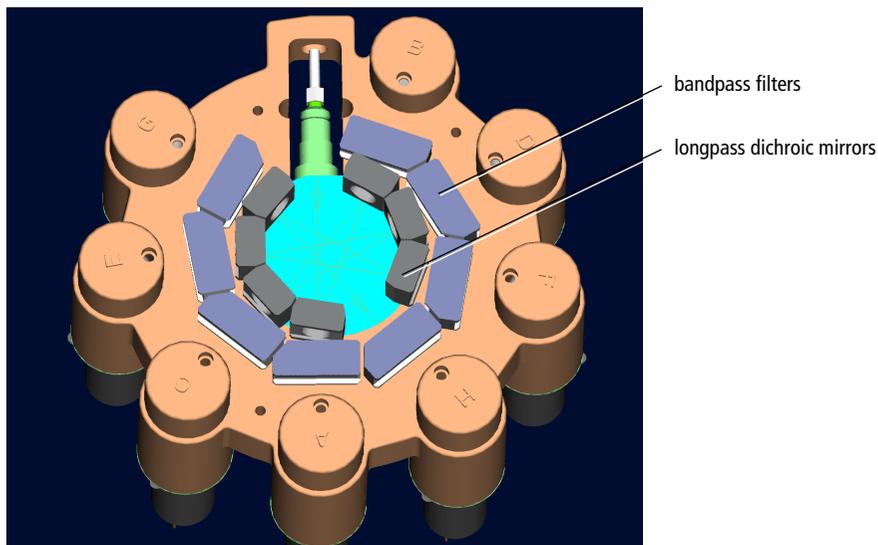
- Shortpass (SP) filters transmit wavelengths that are shorter than the specified value.
- Longpass (LP) filters transmit wavelengths that are longer than the specified value.
- Bandpass (BP) filters pass a narrow spectral band of light by combining the characteristics of shortpass filters, longpass filters, and absorbing layers. Discriminating filters (DF) and ALPHA™ filters (AF) are types of bandpass filters.

When dichroic filters are used as steering optics to direct different color light signals to different detectors, they are called dichroic mirrors or beam splitters.

- Shortpass dichroic mirrors transmit shorter wavelengths of light to one detector while reflecting longer wavelengths to a different detector.
- Longpass dichroic mirrors transmit longer wavelengths to one detector while reflecting shorter wavelengths to a different detector.

The default BD LSR II instrument Octagon and Trigon detector arrays use dichroic longpass mirrors on their inner rings, and bandpass filters on their outer rings. However, you can customize the arrays with other types of filters and mirrors.

In Figure 1-5 on page 33, the inner ring is colored gray, and the outer is blue.



**Figure 1-5** Dichroic filter types in Octagon array

The steering optics and filters mounted on the BD LSR II instrument are listed in Table 2-1 on page 41.

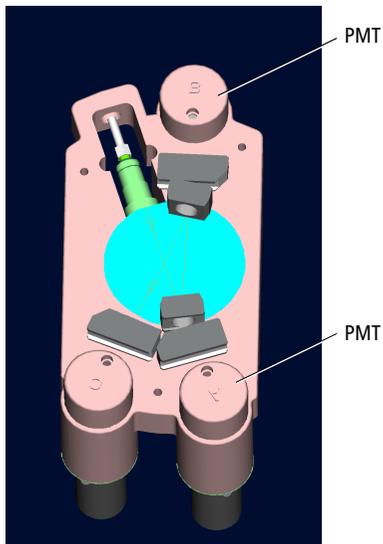
See page 135 for a more detailed explanation of how the filters used in the BD LSR II flow cytometer work.

## Detectors

Light signals are generated as particles pass through the laser beam in a fluid stream. When these optical signals reach a detector, electrical pulses are created that are then processed by the electronics system.

There are two types of signal detectors in the BD LSR II flow cytometer: the photodiode and photomultiplier tubes, or PMTs (Figure 1-6 on page 34). The photodiode is less sensitive to light signals than the PMTs are; thus it is used to detect the stronger FSC signal. PMTs are used to detect the weaker signals generated by SSC and all fluorescence channels. These signals are amplified by applying a voltage to the PMTs. As the voltage is increased, the detector sensitivity increases, resulting in increased signal. As the voltage is decreased, the

detector sensitivity decreases, resulting in decreased signal. Detector voltages are adjusted in BD FACSDiVa software.



**Figure 1-6** PMT-type detectors in Trigon array

The specific, default locations of detectors and filters within BD LSR II instrument Octagon and Trigon arrays are shown in Table 2-1 on page 41.

# Electronics

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Acquisition, analysis, and most BD LSR II instrument functions are controlled by the BD LSR II workstation. It includes a PC, two monitors, and a printer.

Your workstation is equipped with the following:

- a current, BD Biosciences–validated Microsoft Windows operating system
- BD FACSDiVa software, for data acquisition and analysis

Refer to the *BD FACSDiVa User's Guide* for general information.

- supporting documentation for the software



# 2

## Instrument Setup

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- Starting the Flow Cytometer and Computer on page 38
- Setting Up the Optical Filters on page 39
- Preparing Sheath and Waste Containers on page 53
- Preparing the Fluidics on page 57
- Quality Control on page 60

# Starting the Flow Cytometer and Computer

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## 1 Turn on the system.

You can turn on the power to the flow cytometer or the workstation in any order.

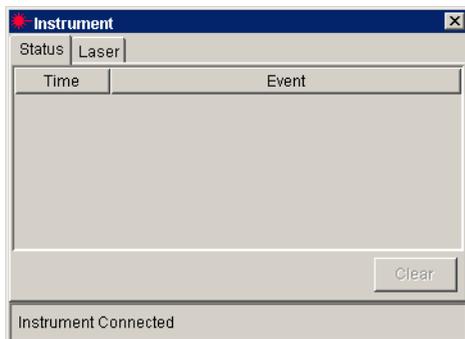
- Turn on the power to the flow cytometer.

For laser warm-up times, see Table 1-1 on page 31.

- Start up the BD LSR II workstation and log in to Windows.

## 2 Launch BD FACSDiVa software by double-clicking the shortcut on the desktop.

## 3 Check the Instrument frame in the software to make sure that the cytometer is connected to the workstation.

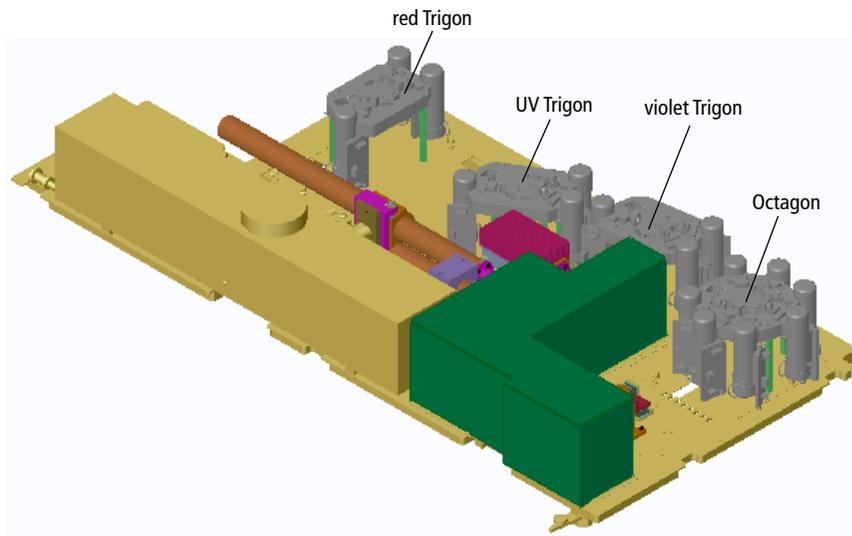


The cytometer automatically connects to the workstation (this can take a few minutes). Once this occurs, the message *Instrument Connected* appears.

If the message *Instrument Disconnected* appears, refer to the troubleshooting section of the *BD FACSDiVa User's Guide*.

## Setting Up the Optical Filters

Before you begin running samples, you should set up the optical filters. In order to do this, familiarize yourself with the location of the required detector arrays by referring to Figure 2-1.



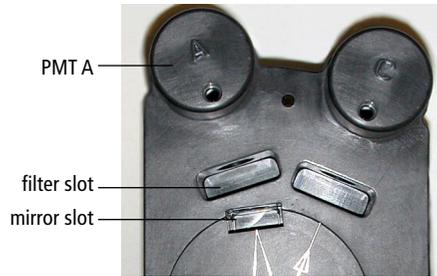
**Figure 2-1** Location of detector arrays beneath the instrument covers of the BD LSR II flow cytometer

Detector Array...	Receives Light From...
Octagon	488-nm blue laser (laser 1)
violet Trigon	405-nm violet laser (laser 2)
UV Trigon	325-nm or 355-nm UV laser (laser 3)
red Trigon	633-nm red laser (laser 4)

# Understanding the Default Filter Configurations

Each PMT usually has two slots in front of it.

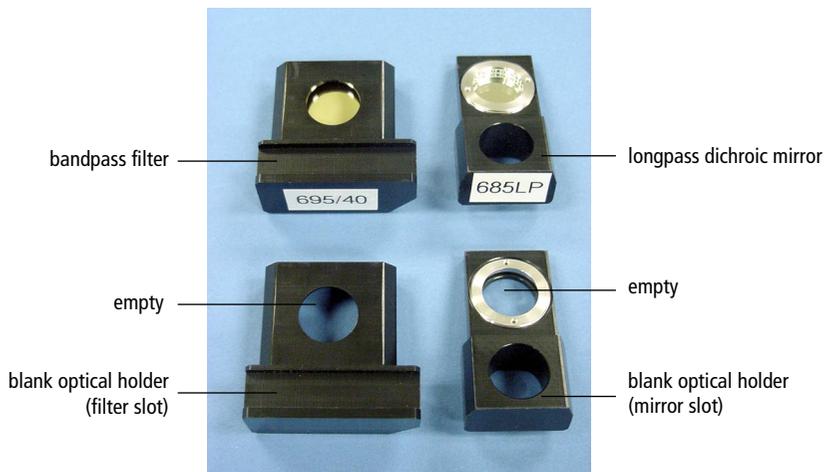
- The slot closest to the PMT holds either a bandpass filter or a blank optical holder.
- The slot furthest from the PMT holds either a longpass dichroic mirror or a blank optical holder.



The last PMT in every detector array has no mirror slot: PMT G (in the Octagon) and PMT C (in all Trignons).

## Blank Optical Holders, Filters, and Mirrors

Blank optical holders look very much like filters and mirrors, but they do not contain any glass in the central opening (Figure 2-2).



**Figure 2-2** Blank optical holders, filters, and mirrors

**NOTE** To preserve data integrity, do not leave any slots unfilled in a detector array when you are using the associated laser. Always use a blank optical holder.

## Default Configurations

The instrument comes with the following filter and mirror configurations. In Table 2-1, *blank* indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a filter or mirror in the PMT position.

**Table 2-1** Default BD LSR II flow cytometer filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome <sup>a</sup>
Octagon (488-nm blue laser)	A	735 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	550 LP	575/26	PE, PI
	D	505 LP	530/30	FITC, GFP
	E	blank	488/10	SSC
	F	blank	blank	N/A
	G	blank	blank	N/A
	H	—	blank	N/A
Violet Trigon (405-nm violet laser)	A	505 LP	525/50	cyan fluorescent protein (CFP), Alexa Fluor® 430
	B	blank	440/40	Pacific Blue™, Marina Blue®, Alexa Fluor 405
	C	—	blank	N/A
UV Trigon (325-nm or 355-nm UV laser; violet option available)	A	505 LP	530/30	indo-1 (blue)
	B	blank	440/40	DAPI, Alexa Fluor 350
	C	—	blank	N/A
Red Trigon (633-nm red laser)	A	735 LP	780/60	APC-Cy7
	B	blank	660/20	APC
	C	—	blank	N/A

- a. A list of all applicable patent numbers appears on page ii (copyright page) of this guide.

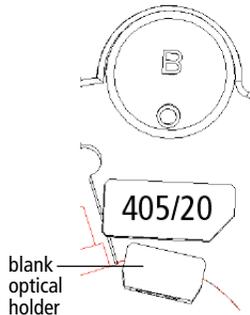
You can view a list of detectors as they pertain to the fluorochromes in the Instrument Configuration dialog box from within BD FACSDiVa software. To view the Instrument Configuration, from the main menu bar choose Instrument > Instrument Configuration.

## Octagon and Trigon Configuration Maps

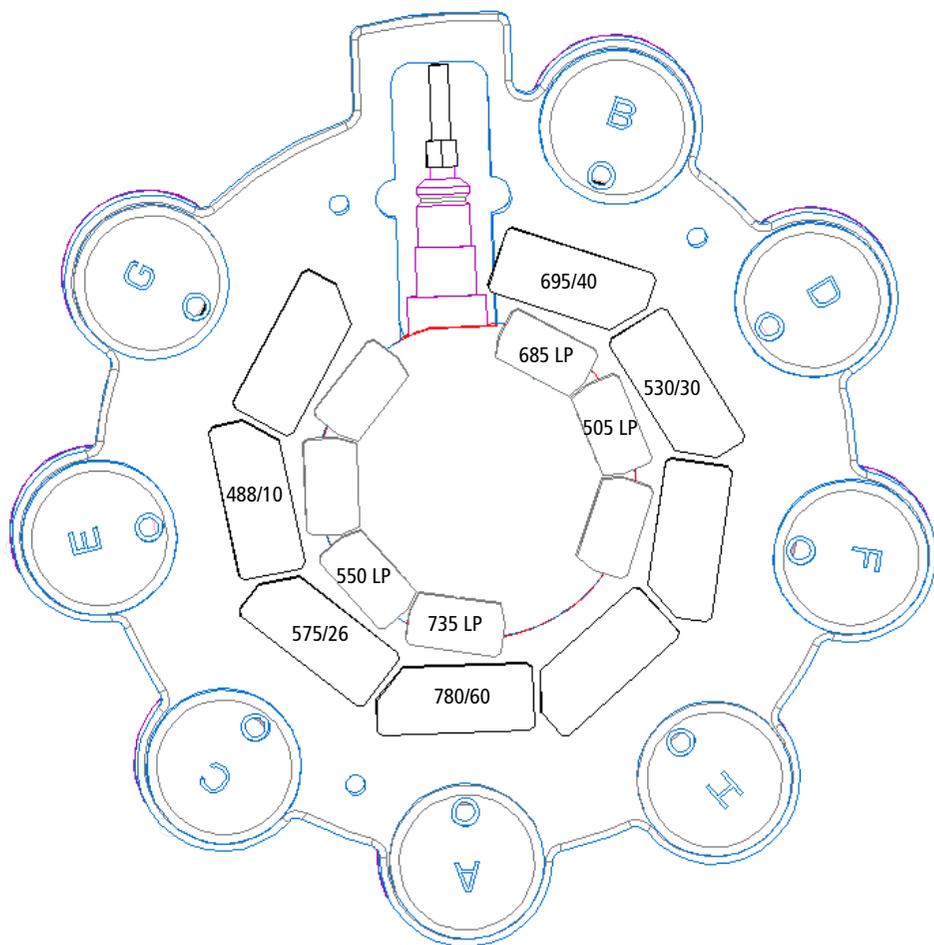
The following sections contain maps of the default Octagon and Trigon filter configurations and common custom filter configurations that can help you set up your instrument.

If a slot is to be filled with a filter or mirror, a number appears on the configuration map. If a slot is to be filled with a blank optical holder, no number is written on the map.

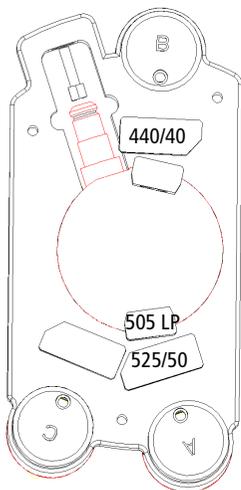
In the following example, bandpass filter 405/20 is in PMT B's filter slot, and a blank optical holder is in PMT B's mirror slot.



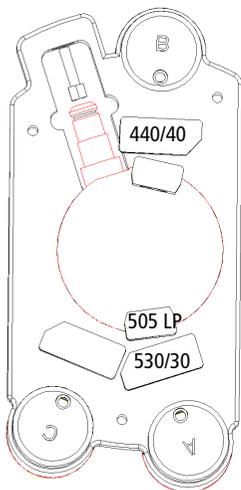
## Default Octagon Configuration (488-nm Blue, Laser 1)



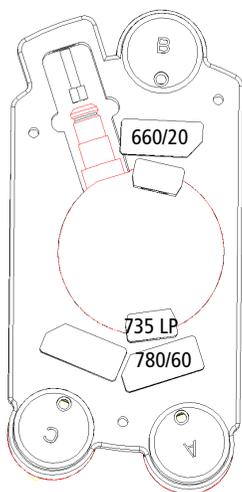
### Default Violet Trigon Configuration (405-nm, Laser 2)



### Default UV Trigon Configuration (325- or 355-nm, Laser 3)



## Default Red Trigon Configuration (633-nm, Laser 4)



## Changing Optical Filters

**⚠ CAUTION** Follow the precautions outlined in Laser Safety on page xv while changing optical filters.

- 1** Lift the appropriate instrument cover.
  - The Octagon array is located under the right instrument cover.
  - The three Trigon arrays are located under the left instrument cover.
- ✓ Tip** To open the left instrument cover, you must first open the right cover and the side door.
- 2** Remove the appropriate filter.
- 3** Replace it with the new filter.

**Tip** The filters and mirrors fit easily into the optical holders in only one way.

**4** Close the instrument cover(s).

## Setting Up Custom Filter Configurations

The following sections show some common custom filter and mirror configurations, and provide diagrams for your ease-of-use. The custom filters used in these diagrams are included with your spares kit.

- Extra Filters and Mirrors (Spares Kit) on page 47
- PE-Texas Red on page 48
- Indo-1 on page 49
- DsRed on page 51
- PerCP or BD Cy-Chrome Reagent on page 52

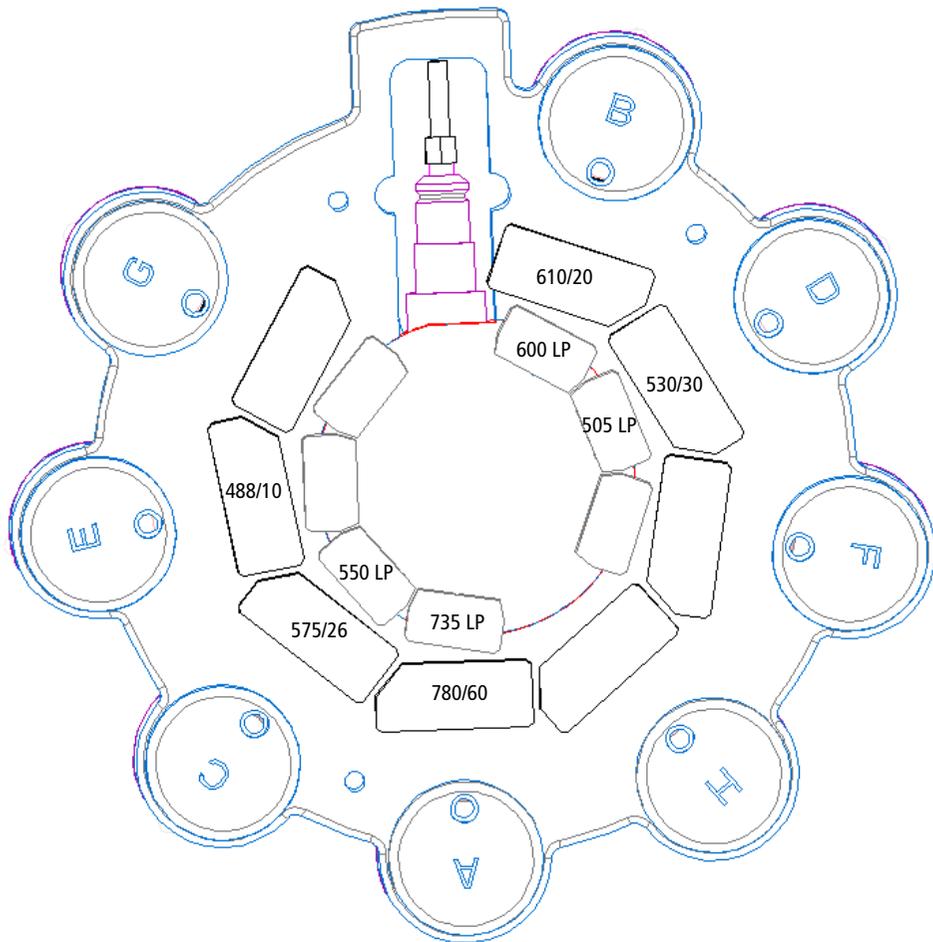
## Extra Filters and Mirrors (Spares Kit)

These filters and mirrors are included in the instrument spares kit.

<b>Detector Array (Laser)</b>	<b>Filters or Mirrors</b>	<b>Fluorochrome</b>
Octagon standard 488-nm blue laser	600 LP	PE-Texas Red™
	635 LP	PerCP, BD Cy-Chrome™ reagent
	670/14	PerCP, BD Cy-Chrome reagent
	610/20	PE-Texas Red
	585/42	DsRed
Violet Trigon optional 405-nm violet laser	None	N/A
UV Trigon optional 325-nm or 355-nm UV laser	450 LP	indo-1
	405/20	indo-1 (violet)
Red Trigon optional 633-nm red laser	None	N/A

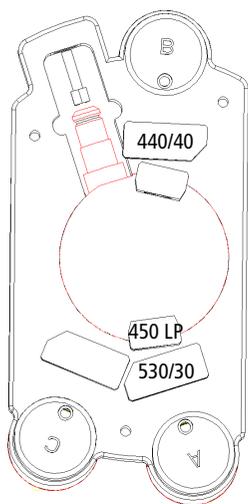
## PE-Texas Red

To use PE-Texas Red, reconfigure only the Octagon as shown. You can leave the Trignons undisturbed.

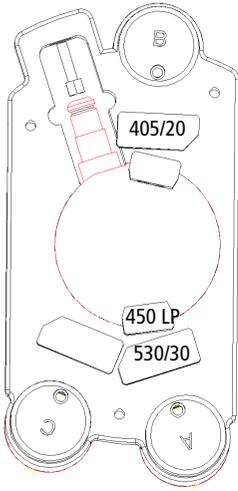


## Indo-1

To use indo-1, reconfigure only the UV Trigon (325- or 355-nm UV laser). You can leave the Octagon undisturbed. If you also have a violet laser, use Figure 2-3. If you do not have a violet laser, use Figure 2-4 on page 50.



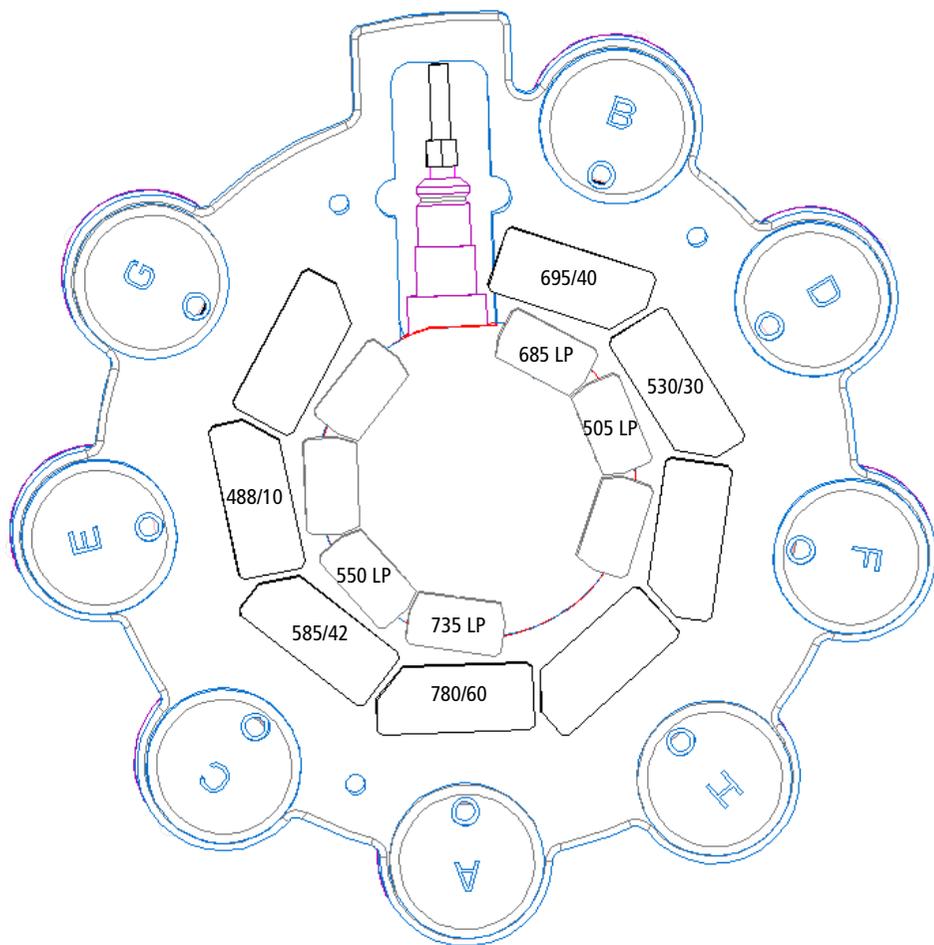
**Figure 2-3** With violet laser option



**Figure 2-4** Without violet laser option

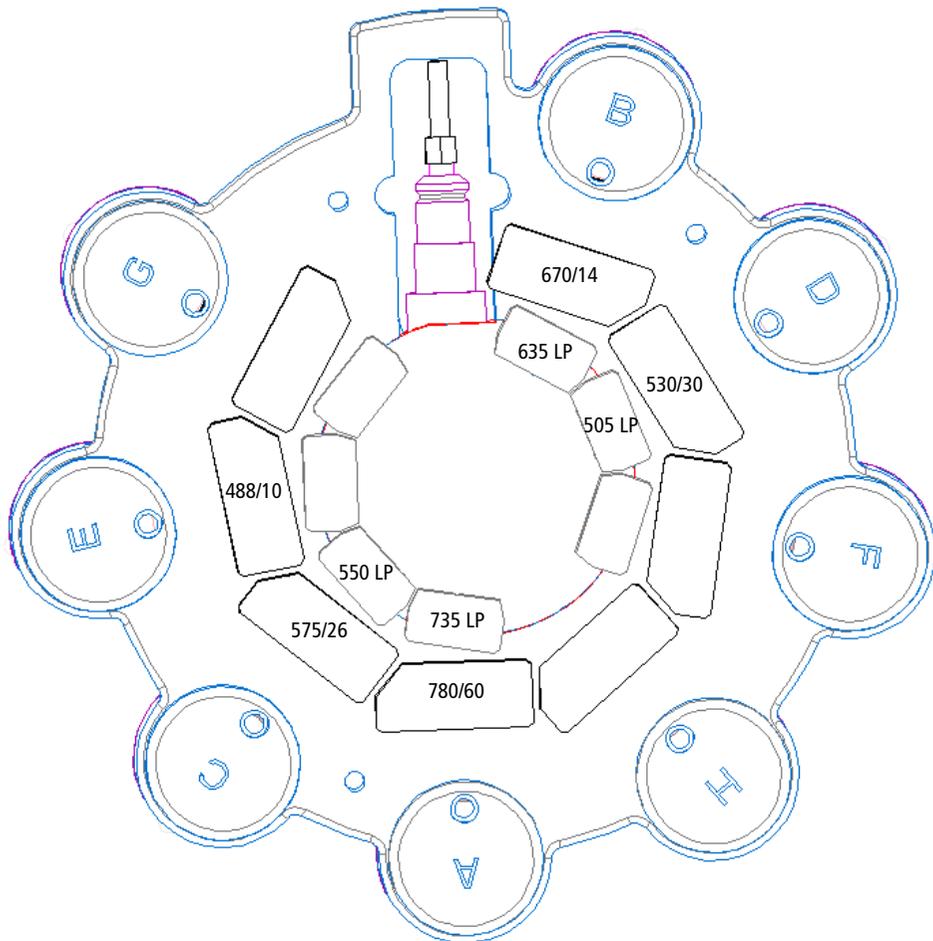
## DsRed

To use DsRed, reconfigure only the Octagon as shown. You can leave the Trignons undisturbed.



## PerCP or BD Cy-Chrome Reagent

To use PerCP or BD Cy-Chrome reagent, reconfigure only the Octagon as shown. You can leave the Trignons undisturbed.



## Filter and Mirror Specifications

**Table 2-2** Longpass dichroic mirrors in Octagon or Trigon

Specification	Measurement
diameter	0.625 in. +0.000, -0.005
minimum clear aperture	0.562 in.
incident angle	11.25°
thickness	0.125 in ±0.005 in.

**Table 2-3** Bandpass filters in Octagon or Trigon

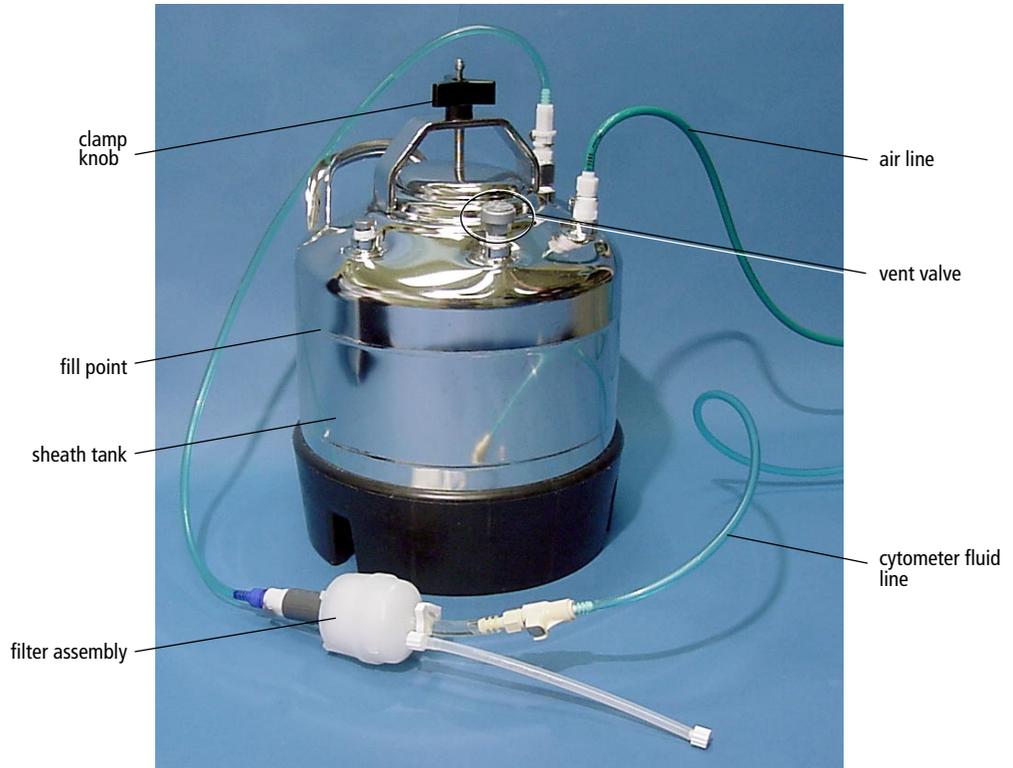
Specification	Measurement
diameter	1.00 in. ±0.010 in.
minimum clear aperture	0.85 in.
incident angle	0°
thickness	0.12–0.35 in.

## Preparing Sheath and Waste Containers

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 **NOTE** Check the fluid levels in the sheath and waste containers every time you use the instrument. This ensures that you will not run out of sheath fluid during an experiment and that the waste container will not become too full.

## Preparing the Sheath Container

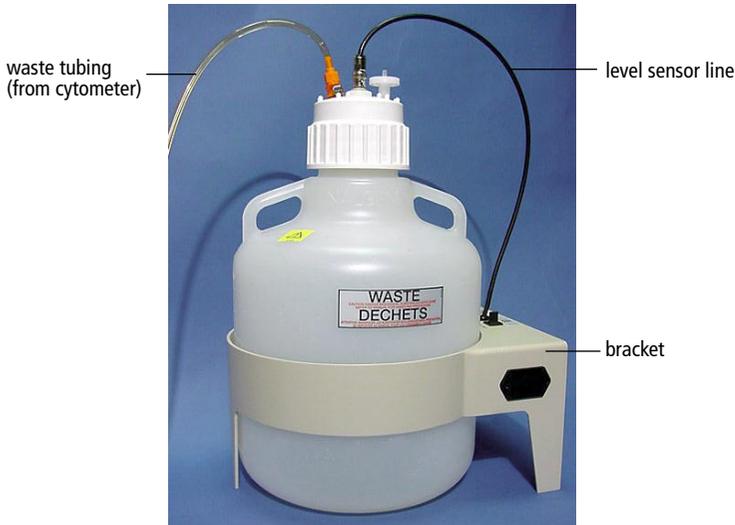


**Figure 2-5** Sheath container

- 1** Make sure that the flow cytometer is in STNDBY mode.
  - 2** Disconnect the air line (green) from the sheath container (see Figure 2-5 on page 54).
  - 3** Depressurize the sheath container by loosening its vent valve.  
Twist the vent valve to loosen it.
  - 4** Remove the sheath container lid.  
Unscrew the clamp knob and lift.
  - 5** Add 6 L of sheath fluid, such as BD FACSTFlow™ solution, to the sheath container.
-  **NOTE** Do not fill the sheath tank to its maximum capacity (8 L). When a full tank is pressurized, erratic instrument performance can result.
-  **Tip** For calcium-flux experiments, use a sheath fluid that does not contain preservatives. For example, use 1X phosphate-buffered saline (PBS).
- 6** Close the sheath container lid.
  - 7** Reconnect the air line (green).
  - 8** Make sure that the lid is finger-tight and that the cytometer fluid line (blue) is not kinked.
-  **Tip** Inspect the sheath container periodically, as sheath fluid can cause corrosion.

## Preparing the Waste Container

**⚠️ WARNING** All biological specimens and materials coming into contact with them are considered biohazardous. Handle as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.



**Figure 2-6** Waste container

- 1** Disconnect the orange waste tubing and the black sensor line from the waste tank.

Keep the lid on the waste tank until you are ready to empty it.

- 2** Empty the waste container.

**⚠️ WARNING** The waste container contents might be biohazardous. Treat contents with bleach (10% of total volume). Dispose of waste using proper precautions and in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

- 3 Add approximately 1 L of bleach to the waste container and close it.
- 4 Reconnect the orange waste tubing and make sure that it is not kinked.
- 5 Reconnect the black sensor line.

## Preparing the Fluidics

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Next, you should make sure the fluidics system is ready. This section covers the following preparations:

- Cleaning the Fluidics (If Necessary)
- Removing Air Bubbles on page 58
- Priming the Fluidics (If Necessary) on page 59

### Cleaning the Fluidics (If Necessary)

If you often use DNA dyes or have samples that tend to clump, you should clean the SIP and flow cell before and after running experiments.

- 1 Move the tube support arm to the side.
- 2 Install a tube containing 3 mL of a 1:10 dilution of bleach on the SIP.  
  
Allow the vacuum to aspirate the bleach solution for approximately 1 minute (1 mL).
- 3 Press the HI sample flow rate button, press the RUN button, and place the tube support arm under the tube.

Allow the bleach to run for 5 minutes.

**4** Repeat steps 1 through 3 with 3 mL each of the following.

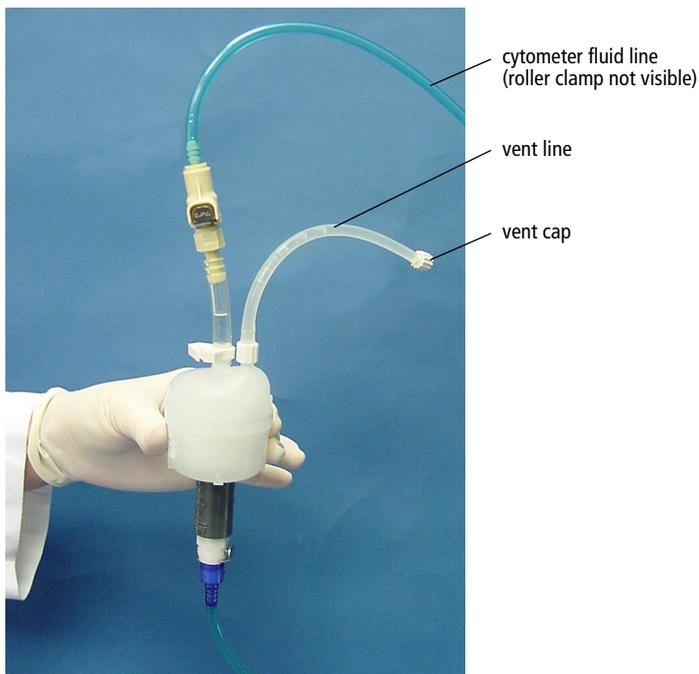
- BD FACSRinse™ detergent
- DI water

## Removing Air Bubbles

Trapped bubbles can occasionally dislodge and pass through the flow cell, resulting in inaccurate data.

**⚠ NOTE** Do not vigorously shake, bend, or rattle the sheath filter or you might damage it.

**1** Check the sheath filter for trapped air bubbles.



**Figure 2-7** Sheath filter

- 2** If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.
- 3** Pinch the vent line closed.
- 4** Loosen the sheath filter vent cap to bleed off any air in the filter; collect the excess fluid in a container.
- 5** Replace the vent cap.
- 6** Check the fluid lines for air bubbles.
- 7** Open the roller clamp on the blue cytometer fluid line (if necessary) to bleed off any air in the lines.
- 8** Close the roller clamp.

## **Priming the Fluidics (If Necessary)**

Sometimes stubborn bubbles are not removed by the previous procedure, or bubbles and debris are stuck in the flow cell. This is indicated by quality-control (QC) results being out of range (see page 60). In these cases, it is necessary to prime the fluidics system.

- 1** Remove the tube from the SIP.
- 2** Press the PRIME fluid control button to force the fluid out of the flow cell and into the waste container.

Once drained, the flow cell automatically fills with sheath fluid at a controlled rate to prevent bubble formation or entrapment. The STNDBY button is yellow after completion.

- 3** Install a 12 x 75-mm tube with 1 mL of DI water on the SIP and place the support arm under the tube.
- 4** Repeat the priming procedure if the QC results are still out of range.

# Quality Control

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An instrument quality control (QC) procedure, performed on a regular basis, provides a standard for monitoring instrument performance. Instrument QC consists of the following:

- running QC samples
- recording parameter means and CVs

QC data should be analyzed for trends consisting of 10 or more runs. By keeping the instrument settings and QC sample constant, changes in the recorded means and CVs will indicate how much instrument performance varies over time.

QC results are affected by laser and fluidics performance. We strongly recommend following the laser and fluidics maintenance procedures in Chapter 6.

See QC Particles on page 158 for a list of acceptable QC beads.

A sample QC log is provided in Appendix B. It can be photocopied or used as a guide in designing your own QC log.

# 3

## Running Samples

---

This chapter describes how to use BD FACSDiVa software to record and analyze sample data.

The following topics are covered in this chapter:

- Performing Sample Optimization Using Instrument Setup on page 62
- Recording and Analyzing Data on page 72

## Before Beginning This Chapter

To perform the steps in this chapter, you should be familiar with the following:

- Instrument startup
- QC
- General software components: workspace components, instrument and acquisition controls, tools for data analysis

Refer to your *BD FACSDiVa Software User's Guide* for a software overview.

# Performing Sample Optimization Using Instrument Setup

---

Sample optimization consists of five main steps; each step is explained in greater detail in the sections that follow. It is important that you perform these steps in the order in which they are listed. You might need to vary certain steps for different sample types.

- 1** Create an Experiment, specify parameters, and add compensation Tubes.
- 2** Adjust the FSC and SSC voltages and the FSC threshold.
- 3** Gate the population of interest.
- 4** Adjust photomultiplier tube (PMT) voltages.
- 5** Calculate compensation.

This section describes how to perform sample optimization using the Instrument Setup feature. Instrument Setup can be used to automatically calculate compensation settings. For more information about this feature, refer to the *BD FACSDiVa Software User's Guide*. If you are performing compensation manually, not all steps will apply.

This section presents an example of sample optimization using a lysed, washed, whole-blood sample (LWB) stained with the following mouse anti-human antibodies.

**Tip** The reagents in boldface are the positive controls.

Unstained control	Mouse IgG <sub>1</sub> FITC/Mouse IgG <sub>1</sub> PE/ Mouse IgG <sub>1</sub> PerCP-Cy5.5/Mouse IgG <sub>1</sub> APC/ Mouse IgG <sub>1</sub> APC-Cy7
FITC-stained control	<b>CD8 FITC</b> /Mouse IgG <sub>1</sub> PE/ Mouse IgG <sub>1</sub> PerCP-Cy5.5/Mouse IgG <sub>1</sub> APC/ Mouse IgG <sub>1</sub> APC-Cy7
PE-stained control	Mouse IgG <sub>1</sub> FITC/ <b>CD8 PE</b> / Mouse IgG <sub>1</sub> PerCP-Cy5.5/Mouse IgG <sub>1</sub> APC/ Mouse IgG <sub>1</sub> APC-Cy7
PerCP-Cy5.5-stained control	Mouse IgG <sub>1</sub> FITC/Mouse IgG <sub>1</sub> PE/ <b>CD8 PerCP-Cy5.5</b> /Mouse IgG <sub>1</sub> APC/Mouse IgG <sub>1</sub> APC-Cy7
APC-stained control	Mouse IgG <sub>1</sub> FITC/Mouse IgG <sub>1</sub> PE/ Mouse IgG <sub>1</sub> PerCP-Cy5.5/ <b>CD8 APC</b> /Mouse IgG <sub>1</sub> APC-Cy7
APC-Cy7-stained control	Mouse IgG <sub>1</sub> FITC/Mouse IgG <sub>1</sub> PE/ Mouse IgG <sub>1</sub> PerCP-Cy5.5/Mouse IgG <sub>1</sub> APC/ <b>CD8 APC-Cy7</b>

# Creating the Experiment

This section describes how to verify the instrument configuration, create a folder and Experiment, specify the parameters for the assay, and add compensation Tubes.

- 1 Choose Instrument > Instrument Configuration and verify the current configuration.

Make sure the configuration lists the parameters to be measured and that the channels correspond to the optical filters. For this example, the Instrument Configuration should include the following parameters: FITC, PE, PerCP-Cy5.5, APC, and APC-Cy7.

Refer to the *BD FACSDiVa User's Guide* for more information.

 **NOTE** For accurate data results, the instrument optics setup must match the current Instrument Configuration.

- 2 Click the corresponding Workspace buttons to display the Browser, Instrument, Inspector, Worksheet, and Acquisition Controls frames, as needed.

- 3 (Optional) Press Ctrl-N to add a new folder to the Browser; rename the folder with your name.

Alternatively, you can name the folder *Sample Optimization* or you can create a Sample Optimization folder inside another folder.

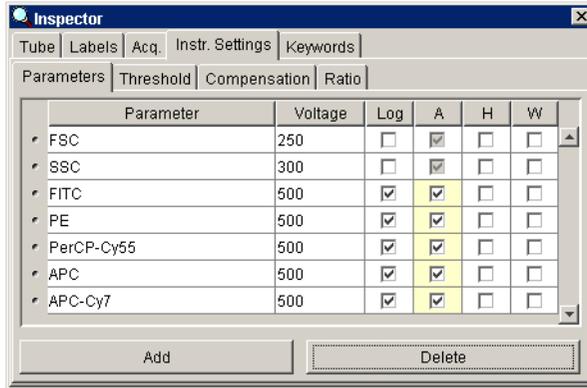
- 4 Press Ctrl-E to create a new Experiment; rename the Experiment with an appropriate name.

For example, use *5-Color Expt*, or your initials followed by an appropriate identifier.

-  **Tip** To place an Experiment inside a folder, select the folder before creating the Experiment.

- 5 Select the Experiment-level instrument settings in the Browser; click on the Parameters tab in the Inspector and delete any unnecessary parameters (Figure 3-1).

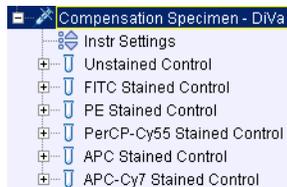
Click the selection button next to the parameter name(s) to select the rows; press the Delete key or click the Delete button in the Inspector.



**Figure 3-1** Parameters for five-color optimization

- 6 Choose Instrument > Instrument Setup > Create Compensation Tubes.

A Compensation Specimen is added to the Experiment, along with a Stained Control Tube for each parameter in the Experiment. Worksheets containing the appropriate plots are added for each compensation Tube.



- 7 (Optional) Create label-specific Tubes, if needed.

Label-specific Tubes are not required for this example; they are needed only when your experiment contains samples stained with the same fluorophore conjugated to different antibodies (labels) that require different

compensation values. This is especially noticeable in tandem conjugates due to lot-to-lot variation. Refer to your *BD FACSDiVa Software User's Guide* for more information about this feature.

## Adjusting the Voltages and Threshold

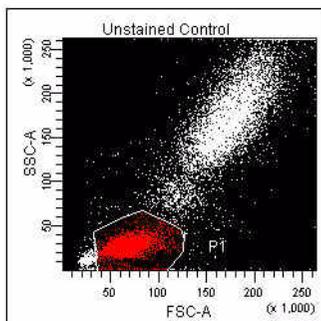
The unstained control will be used to check for nonspecific antibody binding; to adjust forward scatter, side scatter, and FSC threshold; to gate the population of interest (lymphocytes, in this case); and to adjust the PMT voltages.

- 1** Install the unstained control tube on the cytometer.
- 2** Verify that the green Acquisition pointer is in front of the Unstained Control Tube in the Browser; click once on the pointer to begin acquisition.

Alternatively, click the Acquire button in the Acquisition Controls frame.

If the *Start acquisition on pointer change* User Preference is enabled (default option), acquisition starts automatically when the pointer is moved.

- 3** Adjust the FSC and SSC voltages to appropriately display the scatter properties of the LWB sample (Figure 3-2 on page 67).
  - Select the Unstained Control Tube in the Browser.
  - Click the Instr. Settings tab in the Inspector, and then click the Parameters tab.
  - Click the up and down arrows or drag the sliders to adjust the values.



**Figure 3-2** Voltages adjusted

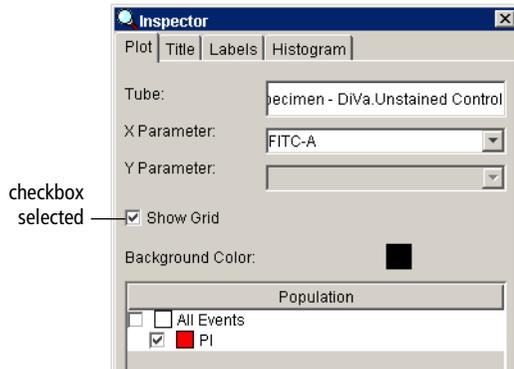
- 4** Click the Threshold tab and adjust the FSC Threshold, if needed.

Set the threshold to remove most of the debris without cutting off the lymphocyte population (Figure 3-2).

- 5** Adjust the P1 gate on the Unstained Control worksheet to surround only the lymphocyte population (Figure 3-2).

Select the gate by clicking on the boundary. Once selected, you can drag the gate to move it, or drag any of the selection handles to change the size and shape of the gate.

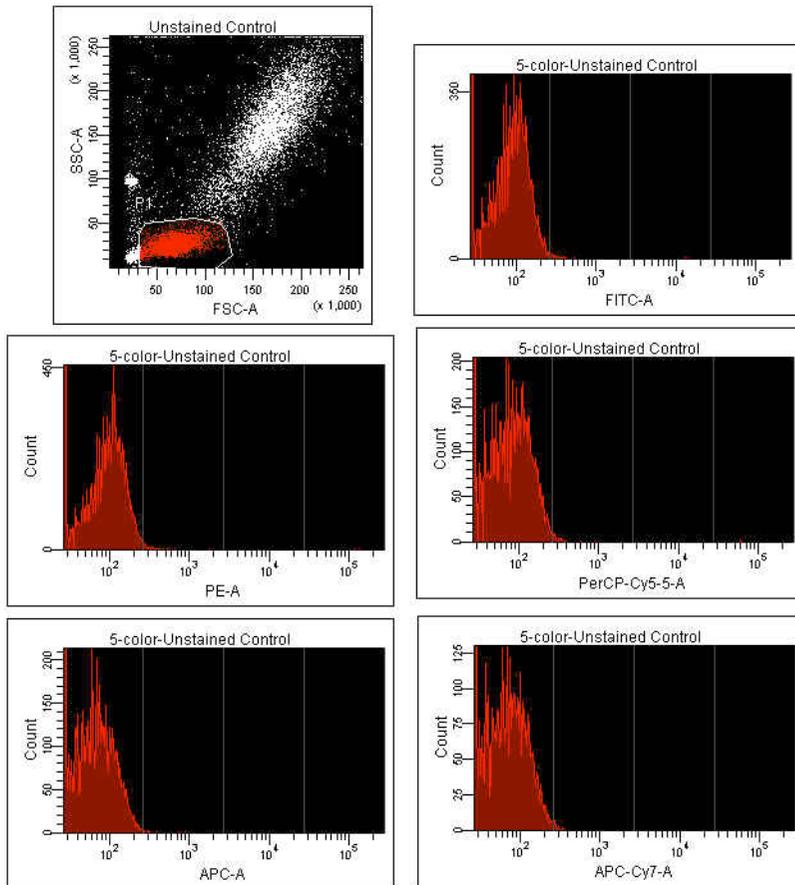
- 6** Select all fluorescence histograms on the Unstained Control worksheet.
- 7** In the Plot Inspector, select the Show Grid checkbox (Figure 3-3 on page 68).



**Figure 3-3** Plot Inspector for fluorescent plots

In a four-log display, values are displayed from 26–262,143. Thus, the first log decade ranges from 26–262. Gridlines are used to delineate log decades on plots.

- 8** Optimize the voltages to place the negative population for each fluorescent parameter within the first log decade (Figure 3-4 on page 69).



**Figure 3-4** Unstained Control Tube after PMT adjustment

**9** Click Record.

When all events have been recorded, remove the unstained control tube from the cytometer.

- Tip** Do not change the PMT voltages after the first compensation Tube has been recorded. In order to calculate compensation, all Tubes must be recorded with the same PMT voltage settings. If you need to adjust the PMT

voltage for a subsequent compensation Tube, you will need to record all compensation Tubes again.

## Calculating Compensation

Before you can calculate compensation, you will need to record data for each single-stained control.

- 1** Install the first stained control tube onto the cytometer.
- 2** Click the Next button; click Acquire, if needed.

Alternatively, move the Acquisition pointer to the next Tube.

If the *Start acquisition on pointer change* User Preference is enabled (default option), acquisition starts automatically when the pointer is moved.

- 3** Adjust the P1 gate to encircle the lymphocytes.
- 4** Ctrl-click the Acquisition pointer to record data.
- 5** When recording is finished, install the next stained control tube onto the cytometer.
- 6** Repeat steps 2 through 5 until data for all stained control tubes has been recorded.

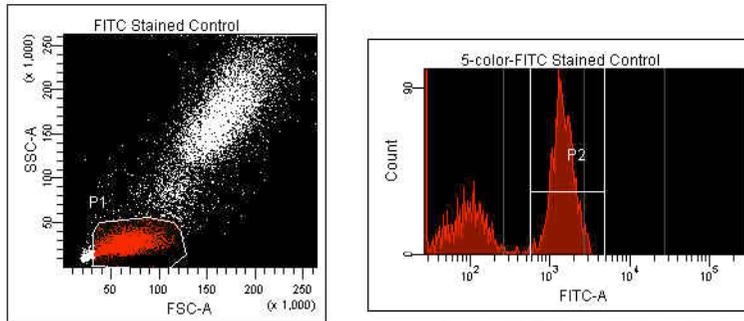
Now that data has been recorded, you need to create regions around the fluorescence-positive populations on the histogram for each stained control Tube.

- 7** Double-click the first Stained Control Tube (FITC Stained Control) to locate the corresponding plots on the worksheet.
- 8** Create an Interval gate around the fluorescence-positive population on the histogram (Figure 3-5 on page 71).

Fluorescence-negative populations were defined using the Unstained Control Tube in the previous section. If negative populations have not

already been defined, you can define them by creating an additional gate around the negative population for each Stained Control Tube.

- ✓ **Tip** Use the Auto-Interval tool to quickly create an Interval gate around the population. To keep the tool selected so you don't have to click the tool for each remaining Tube, hold down the Control key the first time you click the tool. The tool remains selected until you press Esc or select another tool.



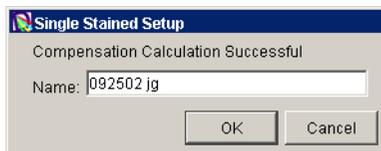
**Figure 3-5** Gating the positive population

- 9 Double-click the next Stained Control Tube in the Browser to locate the corresponding plots on the worksheet.
- 10 Repeat steps 8 and 9 for the remaining compensation Tubes.

Once all regions have been set, you are ready to calculate the compensation.

- 11 Choose Instrument > Instrument Setup > Calculate Compensation.

If the calculation is successful, a dialog box appears where you can enter a name for the compensation Setup.



**12** Enter a name for the compensation Setup; click OK.

**Tip** To keep track of compensation Setups, include the Experiment name, date, or both in the Setup name.

The named Setup is automatically linked to the Experiment's instrument settings.

## Recording and Analyzing Data

---

Once you have optimized the instrument electronics for your sample type, you are ready to record and analyze data.

During analysis, recorded data is displayed in plots, and gates are used to define populations of interest. You can use Acquisition Templates to view and optimize data before it is recorded. BD FACSDiVa software analyzes the gated data and calculates statistics that you can print or export.

This section describes how to use BD FACSDiVa software features to record and analyze sample data. As an example, data will be recorded and analyzed for two samples stained with the following reagent combination: CD4 FITC/CD16+CD56 PE/CD3 PerCP-Cy5.5/CD19 APC/CD8 APC-Cy7.

Two strategies are shown for reusing Analysis objects. If you are running the software on an acquisition workstation (ie, the workstation is connected to the cytometer), you can set up the analysis on an Acquisition Template, and reuse your analysis strategy for multiple samples in a run. Alternatively, Analysis objects can be copied to multiple Tubes at a time.

## Setting Up the Acquisition Template

This section shows you how to use an Acquisition Template to preview and record data for multiple samples.

**1** Create a new Specimen; rename the Specimen *LWB*.

- 2 Create two Tubes under the LWB Specimen; rename the Tubes appropriately.

For example, *T/B/NK\_001* and *T/B/NK\_002*.

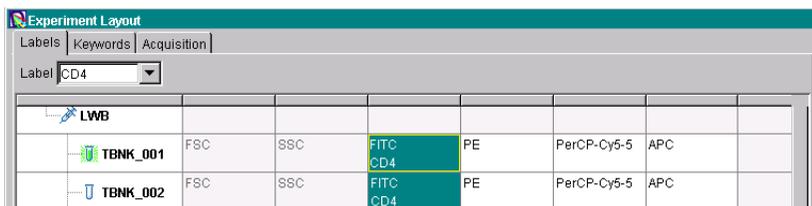
- 3 Create an Acquisition Template; rename the template *Record Data*.

- If the Default Acquisition Template is enabled in User Preferences (default option), the template is already present. Expand the Acquisition Templates folder to locate and rename the template.
- If the Default Acquisition Template preference is disabled, create a template by clicking the Acquisition Template tool in the Browser toolbar. You can create up to ten templates per Experiment.

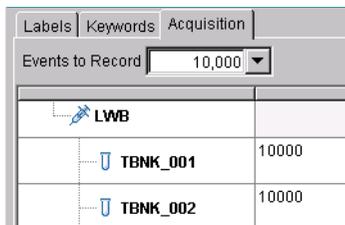
- 4 Use the Experiment Layout dialog box to define labels and to specify the number of events to record for each Tube.

Parameter labels are defined in the Experiment Layout view. Labels will appear on the plot axes and in all statistics views.

- Choose Experiment > Experiment Layout.
- On the Labels tab, enter appropriate labels for the Tube. For example, enter CD4 in the FITC field; use the Tab key to move to the next field.



- On the Acquisition tab, enter 10,000 events for Tubes 001 and 002. Notice that the Acq. tab in the Inspector updates automatically.



- Click OK.

- 5 On the Acquisition Template, create appropriate plots for previewing the data.

To switch to the Template view, double-click the template icon in the Browser or click the Template tool on the Worksheet toolbar.

For example, create FSC vs SSC, FITC vs PE, PerCP-Cy5.5 vs PE, and APC vs APC-Cy7 dot plots.

- Tip** Ctrl-click the Plot tool to keep the tool selected until you create all plots.

## Recording Data

- 1 Install the first sample tube onto the cytometer.
- 2 Move the Acquisition pointer to the first Tube.
- 3 While data is being acquired, draw a region around the lymphocytes; set the other plots to show data from the Lymphocyte population.
- 4 Ctrl-click the Acquisition pointer to record data.
- 5 When all events have been recorded, remove the tube from the cytometer.

- 6 Install the next sample tube onto the cytometer; move the pointer to the corresponding Tube in the Browser.
- 7 Preview the data in the Acquisition Template; Ctrl-click the pointer to record data.

**NOTE** If there are more tubes, repeat steps 5 through 7 until data has been recorded for all of them.

- 8 (Optional) Print the Experiment-level instrument settings.

Right-click the instrument settings icon and choose Print.

## Analyzing Data

This section describes how to set up plots, gates, and a statistics view to analyze the recorded data. By the end of this section, your analysis should look similar to that shown in Figure 3-6 on page 77.

- 1 Create a new Acquisition Template; rename the template *T/B/NK Analysis*.
- 2 Select the first Tube under the LWB Specimen and create the following plots on the Analysis template.
  - FSC vs SSC
  - CD3 PerCP-Cy5.5 vs CD16+56 PE
  - CD3 PerCP-Cy5.5 vs CD19 APC
  - CD3 PerCP-Cy5.5 vs CD8 APC-Cy7
  - CD3 PerCP-Cy5.5 vs CD4 FITC
- 3 Create a Population Hierarchy and a statistics view and move them below the plots on the worksheet.
- 4 Draw a gate around the lymphocytes; use the Population Hierarchy to rename the population *Lymphocytes*.

- 5 Select all plots except the FSC vs SSC plot and specify to show only the Lymphocyte population.
- 6 Select all plots and click the Title tab in the Plot Inspector; select the checkboxes to display the Tube and Population names in the plot titles.



- 7 Edit the statistics view to show only the Lymphocyte population and to display the mean for all fluorochromes.
- 8 Draw a region around the CD3-positive population on the CD3 PerCP-Cy5.5 vs CD16+56 PE plot; name the population *T Cells*.
- 9 Draw a region around the CD16+56-positive population on the same plot; name the population *NK Cells*.
- 10 Draw a region around the CD19 population on the CD3 PerCP-Cy5.5 vs CD19 APC plot; name the population *B Cells*.
- 11 Select the T-cell population in the Population Hierarchy view; draw a region around the double-positive population on the CD3-PerCP-Cy5.5 vs CD8 APC-Cy7 plot, and name the population *T Cytotoxic*.

Because the T-cell population is selected first, the T-cytotoxic cells become a subset of all T cells.

- 12 Select the T-cell population in the Population Hierarchy view; draw a region around the double-positive population on the CD3 PerCP-Cy5.5 vs CD4 FITC plot, and name the population *T Helper*.
- 13 Print the analysis.

See Figure 3-6 on page 77 for an example.

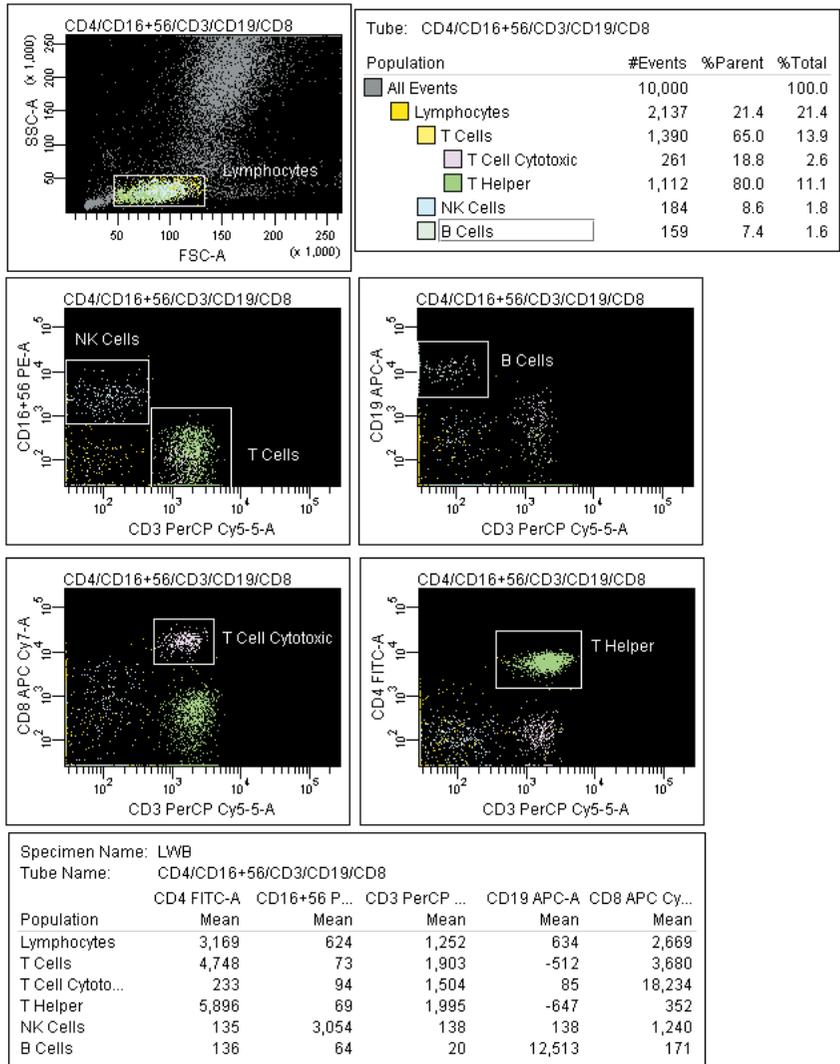


Figure 3-6 Lymphocyte analysis

## Reusing the Analysis

Now that the analysis strategy has been defined, you can use it to analyze the remaining Tubes in the Experiment. Acquisition Templates allow you to apply an analysis strategy to a series of data files without saving the analysis each time. After previewing the data, you can print the analysis or save it to a Tube-specific worksheet (see the following section, Saving the Analysis).

**NOTE** If you set up your Acquisition Template before recording data, you can specify to automatically save the analysis after recording data. This option is set in User Preferences.

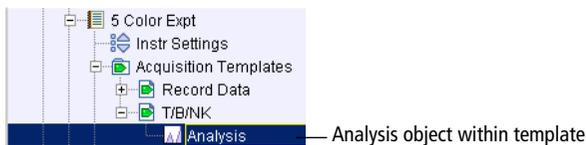
- 1 Move the Acquisition pointer to the next Tube under the LWB Specimen.
- 2 View the data on the Acquisition Template; make adjustments to gates, as needed.

**NOTE** Adjustments will also apply to the next Tube that is viewed on the template. If you don't want to alter the template, save the analysis as described in the next section and make adjustments on the Tube's worksheet.

## Saving the Analysis

Since the Analysis objects were created on an Acquisition Template, the analysis will not be saved with each Tube. If you want to save a copy of the analysis with any Tube, do the following.

- 1 Expand the T/B/NK Analysis Template in the Browser.
- 2 Right-click on the Analysis object and choose Copy.



- 3 Click the Template tool on the Worksheet toolbar () to switch to the Worksheet view.
- 4 Create a new worksheet for the destination Tube; rename the worksheet appropriately.
- 5 Collapse the Tube in the Browser; right-click the Tube icon and choose Paste.

The elements on the template are copied to the new worksheet. You can view the analysis by double-clicking the Tube in the Browser.

- Tip** Apply the analysis to multiple Tubes by selecting more than one Tube before you choose Paste. Enable the *Tube-specific worksheet* user preference to automatically create a new worksheet for the pasted Analysis objects.



# 4

## DNA Analysis

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The following topics are covered in this chapter:

- Criteria for DNA Experiments on page 82
- CEN Optimization on page 83
- CTN Resolution on page 90
- Optimization for Data Recording on page 92

# Criteria for DNA Experiments

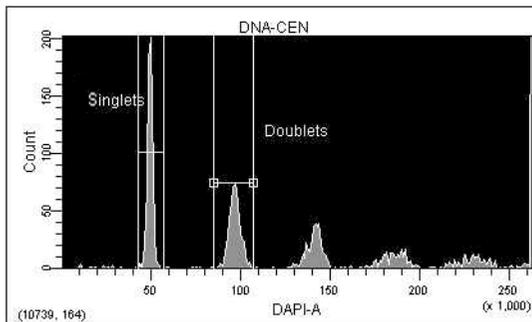
---

In DNA experiments, the flow cytometer must provide the following:

- resolution
- linearity
- ability to distinguish singlets from aggregates

Obtaining good resolution for the DNA signal depends on proper sample preparation and instrument optimization of the optics and fluidics. The resolution of a flow cytometer can be assessed by measuring the CV of a reference particle: the lower the CV, the better the resolution.

Linearity is critical for DNA experiments. To verify the linearity of DNA data, the pulse-area signal is used to measure the amount of DNA fluorescence detected from cells or nuclei. On an instrument that records data linearly, the doublet peak should be located at twice the mean channel of the singlet peak (Figure 4-1).



**Figure 4-1** Area signal and DNA fluorescence

Doublet discrimination, or the ability to resolve singlets from aggregates, is also important for DNA experiments. Aggregated cells or nuclei are detected as events that have two or more times the amount of singlet fluorescence. For cell-cycle analysis, it is important to resolve singlets from aggregates because doublets of  $G_0/G_1$  cells have the same amount of DNA fluorescence as singlet  $G_2+M$  cells.

Therefore, these doublets accumulate in the same fluorescence area channel as singlet G<sub>2</sub>+M cells.

## How To Use DAPI with DNA QC

Before beginning this chapter, do the following.

- Prepare biological standards for instrument quality control using the BD™ DNA QC Particles kit. Substitute the PI solution in the BD DNA QC Particles kit with a 1.0-nanomolar (nM) DAPI solution prepared in 1% BSA.
- Prepare one tube each of chicken erythrocyte nuclei (CEN) and calf thymocyte nuclei (CTN) sample according to the kit instructions. Substitute the DAPI solution for the PI solution.
  - The CEN sample is used to check instrument resolution (CV) and linearity.
  - The CTN sample is used to verify the system's ability to resolve singlets from aggregates.

## How To Use PI with DNA QC

You can use the PI that comes with the BD DNA QC Particles kit instead of DAPI. To do so, prepare the CEN and CTN samples as described in the kit instructions.

## CEN Optimization

---

Use the following procedure to set up BD FACSDiVa software for a DNA experiment that uses DAPI as the DNA-staining dye. If you are using another sample type, modify the steps accordingly. If you are using PI, substitute PI for DAPI throughout the procedure.

# Setting Up the Experiment

- 1 Choose Instrument > Instrument Configuration.
- 2 If you are running BD FACSDiVa software v3.0, select the default LSR II configuration.

If you are running BD FACSDiVa software v2.2.1 or lower, create a configuration for DAPI.

- Click Add Configuration.
- Enter *DAPI* into the Instrument Configuration Name dialog and click OK.
- Select the new DAPI configuration; add the following parameters and assign them to the lasers and detectors shown in Figure 4-2.

Parameter	Laser	Detector
FSC	1	FSC
SSC	1	SSC
DAPI	3	FL8

**Figure 4-2** Parameters, lasers, and detectors for DAPI

- 3 Click Set Configuration.

Make sure you arranged the filters and mirrors in the cytometer to match the software. Use the default Octagon (for SSC) and UV Trigon filter configurations; see Table 2-1 on page 41.

If you are using PI, you will need to create an Instrument Configuration, assigning the PI parameter to Laser 1 (Blue) and Detector FL2 (PMT C). Use the default Octagon filter configuration.

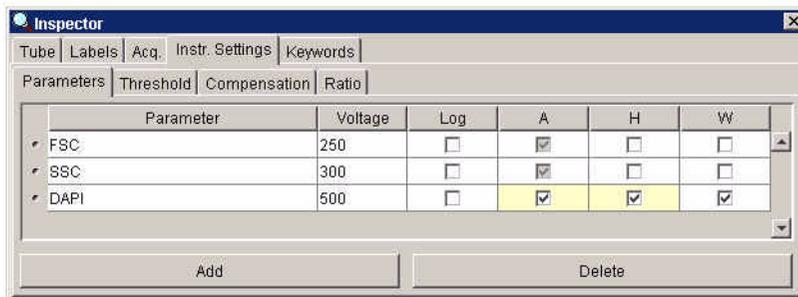
**⚠ NOTE** For accurate data results, the Instrument Configuration dialog box must reflect the physical layout of the BD LSR II Octagons and Trigons. Modifications to the current configuration will not apply unless you click Set Configuration.

- 4 Press Ctrl-E to create a new Experiment; rename the Experiment *DNA*.
- ✓ **Tip** To place the Experiment inside an existing folder, select the folder before creating the Experiment.
- 5 Rename the new Specimen *DNA QC Kit* and rename the first Tube *CEN*.

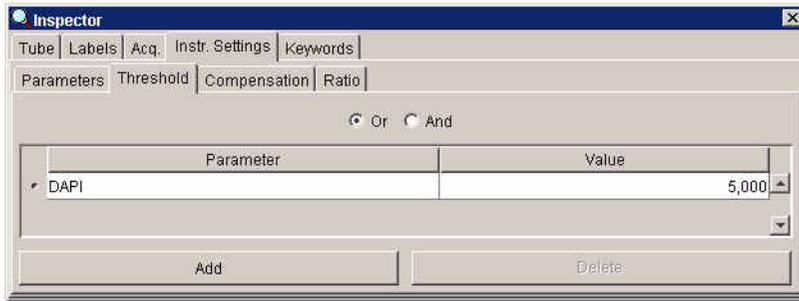
Your Experiment should look similar to that shown in the figure at the right.



- 6 Click the Parameters tab in the Inspector and make the following changes.
  - Delete all parameters except FSC, SSC, and DAPI.
  - Select the Height and Width checkboxes for DAPI.
  - Verify that the Log checkbox is deselected for all parameters.



- 7 Click the Threshold tab and change the threshold parameter to DAPI; verify that the threshold value is set to 5,000.



- 8 Using a Worksheet, create the following plots for the DNA Tube.
- FSC-A vs SSC-A dot plot
  - DAPI-A vs DAPI-W dot plot
  - DAPI-A histogram
- 9 Create a Statistics view and display the mean and CV for DAPI-A and DAPI-W.
- Right-click the plot and choose Create Statistics View.
  - Click the Edit Statistics button in the Inspector.
  - On the Population tab, deselect # Events and %Parent.
  - On the Statistics tab, select the Mean and CV for DAPI-A and DAPI-W (Figure 4-3 on page 87).
  - Set Decimal Places to 1 for the CVs.

Parameters	All	Median	Geomet...	Mean	CV	Max	Min	SD	Mode
FSC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
BSC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
DAPI-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
DAPI-W	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
DAPI-H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Time	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Decimal Places		0	0	0	1	0	0	0	0

**Figure 4-3** Setting up the Statistics view

- In the Acquisition Controls frame, set the Number to Record to 10,000 evt and the Events to Display to 500 evt.



- Tip** Decreasing the number of displayed events will increase the data refresh rate.

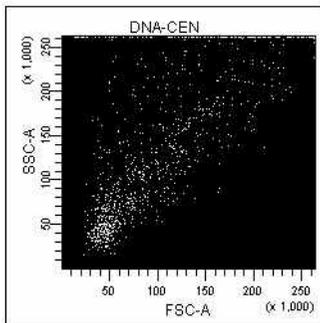
## Running CEN

- On the control panel, press RUN and LO.
- Install the CEN sample tube on the SIP.
- Verify that the green Acquisition pointer is in front of the CEN Tube in the Browser; click once on the pointer to start acquisition.

Events appear in the plots.

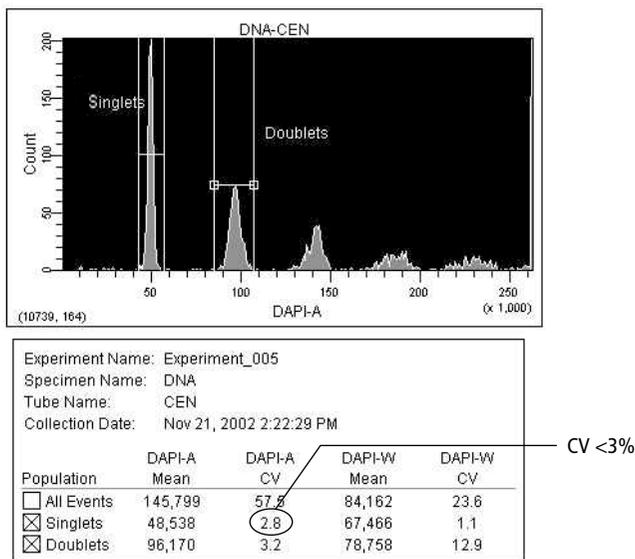
The event rate is displayed in the Acquisition Status frame.

- 4 Adjust the FSC and SSC voltages to place the CEN on scale in the FSC vs SSC dot plot.



**Figure 4-4** FSC and SSC voltages adjusted

- 5 Adjust the DAPI voltage to place the singlet nuclei at approximately channel  $50 \times 10^3$  on the DAPI-A axis in the DAPI-A histogram plot.
- 6 Adjust the event rate to approximately 200 events/second with the Sample Fine Adjust knob.
- 7 Draw an Interval gate around the first two peaks on the DAPI-A histogram; name the populations *Singlets* and *Doublets* (Figure 4-5 on page 89).



**Figure 4-5** Defining Singlet and Doublet populations

**8** Check the CV of the Singlet population.

- If the CV is  $\leq 3\%$ , continue with step 9.
- If the CV is  $> 3\%$ , decrease the flow rate with the Sample Fine Adjust knob. If the CV does not improve, see Troubleshooting on page 121.

**9** Click Record to save the data.

**10** Check the linearity and print the worksheet.

- Note the means of the Singlet and Doublet populations. Divide the mean of the Doublets by the mean of the Singlets. The Doublet/Singlet ratio should be  $2.00 \pm 0.05$ . If you cannot achieve a ratio between 1.95–2.05, contact BD Customer Support.
- Copy the means, CVs, and the calculated linearity result into the QC log.

- 11 Remove the CEN sample from the cytometer.

## CTN Resolution

---

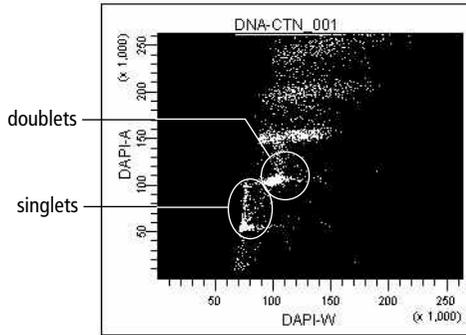
Singlets can be distinguished from aggregates based on size. With BD FACSDiVa software, aggregates can be resolved from singlets on an Area vs Width plot. On the Area vs Width plot, singlets are distinguished from doublets by the Width measurement; singlets have a smaller Width measurement. Discriminating the singlets from the aggregates enhances the accuracy of cell-cycle analysis.

### Running CTN

- 1 On the control panel, press RUN and LO.
- 2 Install the CTN sample tube on the cytometer.
- 3 Adjust the event rate to approximately 500 events/second.
- 4 Click the Next button in the Acquisition Controls frame; change the name of the new Tube to *CTN*.



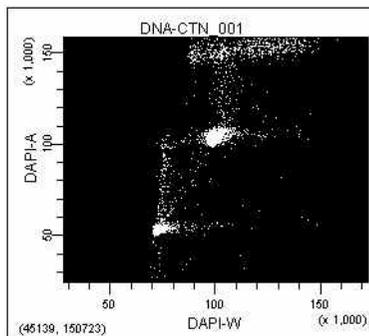
The Next button duplicates the CEN Tube and Analysis objects. The new plots and Statistics view appear below the previous objects on the worksheet. Acquisition starts automatically and events appear in the plots. (Figure 4-6 on page 91).



**Figure 4-6** Unresolved singlets and doublets in unzoomed plot

- 5** Adjust the DAPI voltage to place the first peak at approximately channel  $50 \times 10^3$  on the DAPI-A axis.
- 6** (Optional) Use the Zoom-In tool to magnify the area showing the singlets and doublets on the DAPI-A vs DAPI-W plot.

Magnify the area from the left of the singlets to the right of the doublets, including only the area of interest.



**Figure 4-7** Doublet discrimination in zoomed-in plot

- 7** (Optional) Draw a gate around the singlet CTN.

- 8** Adjust the event rate to approximately 200 events/second with the Sample Fine Adjust knob.
- 9** Click Record to save the data; print the worksheet.
- 10** Remove the CTN Tube from the cytometer; put the instrument in Standby.

## Optimization for Data Recording

---

Optimize the instrument settings for the actual sample.

- 1** Install the sample tube and optimize the FSC and SSC voltages.
- 2** Optimize the DAPI voltage to place the singlets close to channel  $50 \times 10^3$ .
- 3** Verify the doublet discrimination on the DAPI-A vs DAPI-W plot.

After optimizing the instrument settings, record data for each sample tube. Export data files for analysis in a third-party application such as ModFit *LT*<sup>™</sup>.

# 5

## Calcium Flux

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The following topics are covered in this chapter:

- Intracellular Calcium Concentration on page 94
- Calcium Flux Optimization on page 95
- Measuring Calcium Flux on page 101

# Intracellular Calcium Concentration

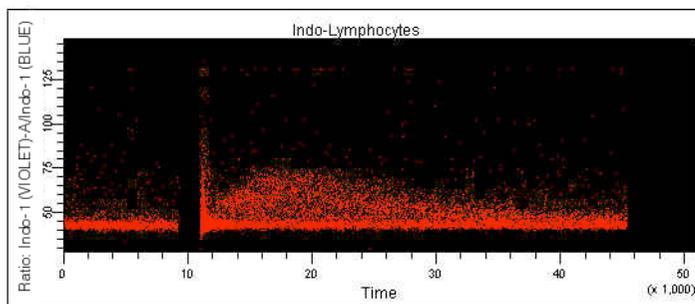
---

Flow cytometry can be used to measure the concentration of intracellular free calcium ions. Measurement of calcium ion ( $\text{Ca}^{++}$ ) concentration can be made on large numbers of single cells, which provides information about the number of responding cells as well as the relative magnitude of the response to a given stimulus.  $\text{Ca}^{++}$  concentration can be correlated with other parameters, such as time, phenotype, and cell cycle.

In their resting state, eukaryotic cells maintain an internal  $\text{Ca}^{++}$  concentration far less than that of the extracellular environment. Elevation in intracellular  $\text{Ca}^{++}$  concentration is often used as an indicator of cellular activation in response to a stimulus. Calcium flux is also an indicator of whether the cells in a population remain functional after exposure to a drug or other compound.

Several fluorescent dyes measure intracellular  $\text{Ca}^{++}$  levels. For most of them, the amount of  $\text{Ca}^{++}$  entering a cell is indicated by a change in fluorescence emission. For example, the emission spectrum of indo-1 changes from blue to violet upon binding to  $\text{Ca}^{++}$ . The ratio of violet to blue fluorescence is independent of the amount of dye within the cell.

When normal cells are analyzed for calcium flux with indo-1 by flow cytometry, a shift in the violet/blue ratio is obtained (Figure 5-1). A break in data occurs when the stimulus is added to the sample tube. The increase in the ratio over time reflects the increase in intracellular  $\text{Ca}^{++}$  concentration.



**Figure 5-1** Calcium flux data

# Calcium Flux Optimization

---

Before beginning this section, do the following:

- Start up the instrument and perform QC.
- Ensure that the appropriate filters are installed. See *Setting Up the Optical Filters* on page 39.
- Review the following section, *Using the Time Parameter*.

## Using the Time Parameter

The Time parameter shows how events change over time. In calcium flux experiments, the Time parameter displays the rate at which the cells in the sample respond to a stimulus.

The Time parameter is a fixed scale and cannot be altered. The values for the Time parameter are in 10-ms increments; thus for a given event, a Time parameter of 123 represents 1,230 ms. Because a plot spans 2.6–262,143, tickmarks will span 2.6–2,621,430 ms (or 44 minutes). Therefore, it takes an event approximately 44 minutes to travel from one end of a plot to the other.

- Tip** To allow enough time for Ca<sup>++</sup> flux response and resolution, enter a large value for the *Events to record* before recording events. You cannot enter a specific time in which to record events or assign a time resolution.
- Tip** Do not restart data recording during a calcium flux experiment.

## Setting Up the Experiment

- 1 Choose Instrument > Instrument Configuration and verify the current configuration.

Make sure that the configuration lists the Indo-1 (VIOLET) and Indo-1 (BLUE) parameters, and that the lasers and channels correspond to

the optical filters in the instrument. You can find a configuration map for indo-1 on page 49.

**NOTE** For accurate data results, the Instrument Configuration dialog box must reflect the filters currently installed in the BD LSR II flow cytometer.

**2** Press Ctrl-E to create a new Experiment; rename the Experiment *Calcium Flux*.

**Tip** To place the Experiment inside an existing folder, select the folder before creating the Experiment.

**3** Rename the first Tube *Ca 1*.

Your Experiment should look similar to that shown in the figure at the right.

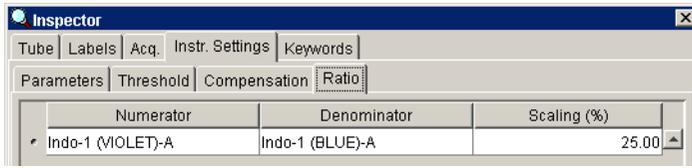


**4** Click the Parameters tab in the Inspector and make the following changes.

- Delete all parameters except FSC, SSC, Indo-1 (BLUE), and Indo-1 (VIOLET).
- Verify that the Log checkbox is deselected for all parameters.

Inspector						
Tube   Labels   Acq.   Instr. Settings   Keywords						
Parameters   Threshold   Compensation   Ratio						
Parameter	Voltage	Log	A	H	W	
▾ FSC	526	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
▾ SSC	284	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
▾ Indo-1 (BLUE)	500	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
▾ Indo-1 (VIOLET)	500	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

- 5 Click the Ratio tab and click the Add button; choose Indo-1 (VIOLET)-A for the Numerator and Indo-1 (BLUE)-A for the Denominator.



- 6 Create the following dot plots for the Ca 1 Tube.

- FSC-A vs SSC-A
- Indo-1 (VIOLET) vs Indo-1 (BLUE)
- Time vs Ratio: Indo-1 (VIOLET)/Indo 1 (BLUE)

- 7 Show the Tube and Population names in the plot titles.

Select all plots on the worksheet, click the Title tab in the Inspector, and select the appropriate checkboxes (Figure 5-2 on page 97).

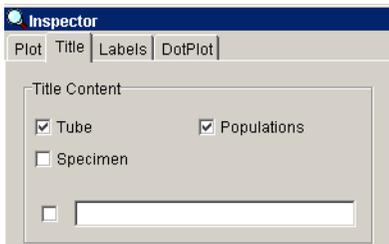


Figure 5-2 Showing Tube and Population names in plot titles

- 8 Create a Statistics view and display the mean for the UV parameters and the ratio.
  - Right-click the Ca 1 Tube and choose Create Statistics View.
  - Select the Statistics view, and click the Edit Statistics View button in the Inspector.
  - Add the required statistics; delete #Events and %Parent from the Population tab.
- 9 In the Acquisition Controls frame, set the Events To Record to 1,000,000 evts and the Events To Display to 500 evts.



Only the specified number of events is displayed in plots during acquisition and recording. After data recording is complete, all recorded events will be displayed.

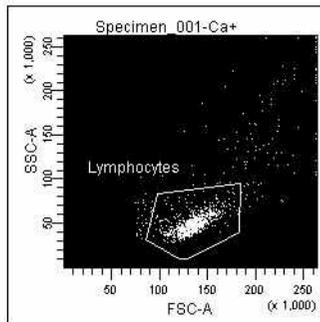
## Optimizing the Calcium Sample

- 1 On the control panel, press RUN and LO.
- 2 Install the unstimulated sample on the SIP.
- 3 Verify that the green Acquisition pointer is in front of the Ca 1 Tube in the Browser; click once on the pointer to start acquisition.

Events appear in the plots.

The event rate is displayed in the Acquisition Status frame.

- 4 While viewing the FSC vs SSC plot (Figure 5-3), make the following adjustments.
- Adjust the FSC and SSC voltages to place the sample on scale in the FSC vs SSC dot plot.
  - Adjust the FSC threshold to remove debris without cutting into the population of interest.
  - Draw a gate around the lymphocytes; use the Population Hierarchy view to rename the population *Lymphocytes*.

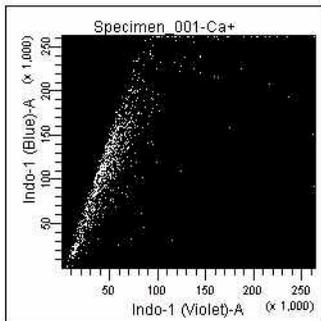


**Figure 5-3** Adjusting FSC and SSC voltages

- 5 Format the remaining two dot plots to show the Lymphocyte population.
- Select the two plots, right-click inside one of the plots and choose Show Populations > Lymphocytes.

- 6** Adjust the Indo-1 (VIOLET)-A and the Indo-1 (BLUE)-A voltages to optimize the signal.

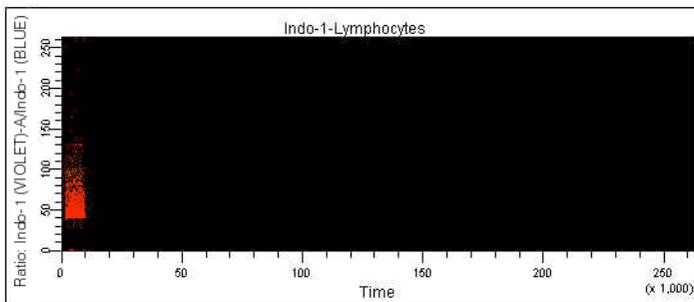
The signal should extend along the Indo-1 (BLUE) axis and should be slightly off the baseline for both axes.



- 7** Adjust the Ratio Scaling to set the baseline between 0–50,000.

Select the Tube in the Browser and click the Instr. Settings > Ratio tab in the Inspector.

To adjust the setting, select the value in the Scaling field, enter a new value, and press Enter. Repeat as needed to achieve the required results.



# Measuring Calcium Flux

---

Do the following to record data for a calcium flux experiment.

- 1** Change the Events to Display to 50,000 events.
- 2** Verify that the unstimulated sample is still running; adjust the event rate to approximately 200 events/second.

The event rate is displayed in the Acquisition Status frame.

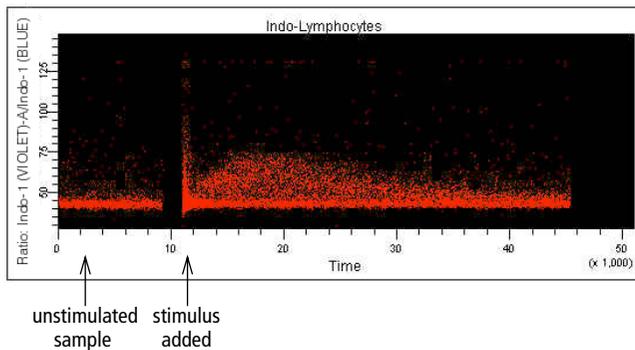
- 3** Click Record.
- 4** When approximately 10,000 events have been recorded, remove the unstimulated sample tube from the cytometer.

Keep the instrument in RUN mode as you do this.

 **NOTE** Do not stop recording or acquisition or the data display will reset to zero.

- 5** Add the stimulus to the tube and mix thoroughly.
- 6** Reinstall the tube on the SIP.

After a few seconds, the  $\text{Ca}^{++}$  concentration begins to increase on the Time vs Ratio plot (Figure 5-4 on page 102).



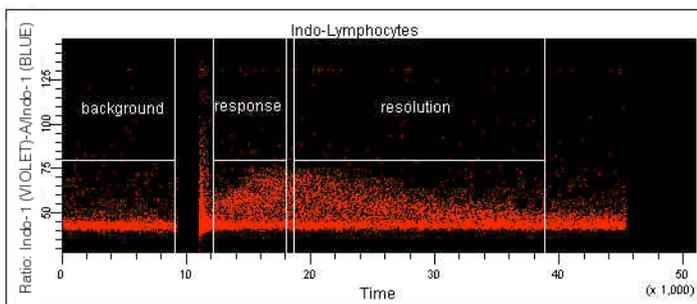
**Figure 5-4** Cellular response to stimulus over time

- 7** Click the Acquisition pointer when the cells are no longer reacting to the stimulus.
- 8** Remove the tube from the cytometer.
- 9** Clean the fluidics system with 10% bleach for 5 minutes, and then with DI water for 5 minutes.
- Tip** Make sure to remove any remaining stimulus that would activate cells in subsequent samples.
- 10** To run another sample, install the next sample tube onto the cytometer.
- 11** Click the Next button; rename the new Tube as appropriate.
- 12** Repeat steps 3 through 10.

## Analyzing Data

**Tip** You can export the data for analysis in an appropriate third-party application.

- 1 To better visualize the cellular response, draw a series of Interval gates on the Time vs Ratio dot plot (Figure 5-5).



**Figure 5-5** Calcium flux data points over time

- 2 Use the Statistics view to display the increase in the Ratio mean over time.



# 6

## Maintenance

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- Daily Cleaning and Shutdown on page 106
- Scheduled Maintenance on page 109
- Periodic Maintenance on page 114

The BD LSR II instrument is designed to require minimum maintenance. However, to preserve the reliability of the instrument, you must regularly perform basic preventive maintenance procedures. This chapter explains routine cleaning procedures you should follow to keep your instrument in good condition.

 **WARNING** All biological specimens and materials coming into contact with them are considered biohazardous. Handle as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

## Daily Cleaning and Shutdown

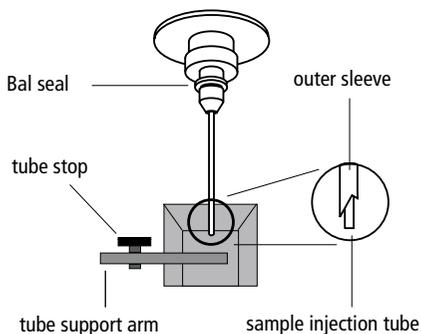
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Perform the following maintenance procedures every day:

- Daily Fluidics Cleaning on page 107
- Daily Shutdown on page 108

## Daily Fluidics Cleaning

Each time you shut down the instrument, clean the sample injection tube and the area between the injection tube and the outer sleeve (Figure 6-1). This prevents the sample injection tube from becoming clogged and removes dyes that can remain in the tubing.



**Figure 6-1** Sample injection port (SIP)

**⚠ NOTE** A 5% solution of sodium hypochlorite can be substituted for undiluted bleach in the following cleaning procedures; however, higher concentrations of sodium hypochlorite and use of other cleaning agents might damage the instrument.

- 1 Move the tube support arm to the side.
- 2 Install a tube containing 3 mL of a 1:10 dilution of bleach on the SIP.  
  
Allow the vacuum to aspirate for approximately 1 min (1 mL of the bleach solution).
- 3 Press the HI sample flow rate button, and place the tube support arm under the tube.  
  
Allow the bleach to run for 5 minutes.
- 4 With the tube support arm moved to the side, install a tube containing 3 mL of BD FACSRinse solution on the SIP.

Allow the vacuum to aspirate approximately 1 min (1 mL of the BD FACSRinse solution).

- 5** Place the support arm under the tube and allow the BD FACSRinse solution to run for 5 minutes.
- 6** Repeat step 4 and step 5 with DI water.
- 7** Leave a tube containing 1 mL of DI water on the SIP with the support arm under the tube.

**⚠ NOTE** Do not leave more than 1 mL of water on the SIP. When the BD LSR II flow cytometer is turned off or left in STNDBY mode, a small amount of fluid will drip back into the sample tube. If there is too much fluid in the tube, it could overflow and affect instrument performance.

## Daily Shutdown

- 1** Set the cytometer to STNDBY mode.
- 2** Turn off the flow cytometer.

**⚠ NOTE** If the instrument will not be used for a week or longer, perform a system flush (see page 109) and leave the fluidics system filled with DI water to prevent saline crystals from clogging the fluidics.

# Scheduled Maintenance

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Perform the following maintenance as indicated in Table 6-1.

**Table 6-1** Scheduled maintenance timetable

Procedure	Schedule	Page
System Flush	once every 2 weeks	page 109
Laser Maintenance	once every week	page 110
Waste Management System Maintenance	once every 2 weeks	page 110

## System Flush

An overall fluidics cleaning is required to remove debris and contaminants from the sheath tubing, waste tubing, and flow cell. Perform the system flush at least every two weeks.

 **WARNING** Instrument hardware might be contaminated with biohazardous material. Use 10% bleach to decontaminate the BD LSR II flow cytometer. Flushing with 10% bleach is the only procedure recommended by BD Biosciences for decontaminating the instrument.

**1** Remove the sheath filter (see Changing the Sheath Filter on page 114).

 **NOTE** Do not run detergent, bleach, or ethanol through the sheath filter. They can break down the filter paper within the filter body, causing particles to escape into the sheath fluid, possibly clogging the flow cell.

**2** Empty the sheath container and rinse it with DI water.

**3** Fill the sheath container with at least 1 L of a 1:10 dilution of bleach or full-strength BD™ FACSClean solution.

**4** Empty the waste container if it is full.

- 5** Install a tube with 3 mL of a 1:10 dilution of bleach or full-strength BD FACSClean solution on the SIP.
- 6** Press the RUN fluid control button; run the cytometer on HI for 30 minutes.
- 7** Press the STNDBY fluid control button and depressurize the sheath container by lifting the pressure relief valve cap.
- 8** Empty the sheath container and rinse it with DI water.
- 9** Fill the sheath container with BD FACSRinse solution.
- 10** Install a tube with 3 mL of full-strength BD FACSRinse solution on the SIP.
- 11** Repeat steps 6 through 8 with BD FACSRinse solution.
- 12** Fill the sheath container with at least 1 L of DI water.
- 13** Repeat steps 4 through 8 with DI water.
- 14** Replace the sheath filter and refill the sheath container with sheath fluid.

## **Laser Maintenance**

To extend the life of the HeCd laser, turn on the instrument for 3–4 hours at least once per week.

## **Waste Management System Maintenance**

The waste management system for the BD LSR II instrument has an alarm powered by a 9-volt battery that must be tested and changed regularly to ensure its continued operation. Test the battery every two weeks after you flush the system. Change the battery as needed.

## Testing the Battery and Alarm

- 1 Locate the Battery Test switch on the waste container bracket.

Figure 6-2 shows a top view of the bracket.



**Figure 6-2** Battery Test switch

- 2 Toggle the switch.

If the battery and the alarm are working properly, you should hear buzzing. If you do not hear any sound, change the battery as described in the following section.

- 3 Release the switch.

## Changing the Battery

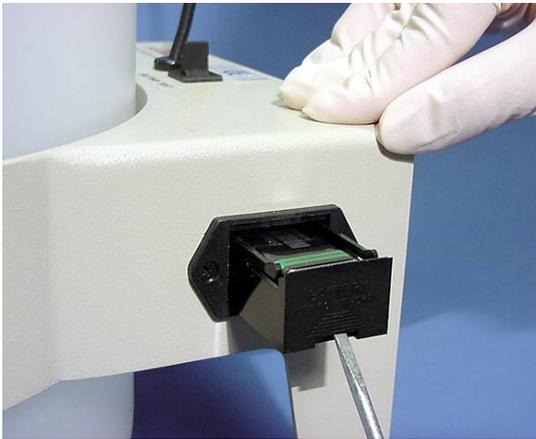
You will need the following supplies to change the battery:

- small flat head screwdriver
- 9-volt battery

Perform the following steps.

- 1** Insert the tip of a flat-head screwdriver into the slot as shown in Figure 6-3 and pry it out.

A small drawer will slide out.



**Figure 6-3** Opening drawer

- 2** Remove the drawer.

- 3 Remove the battery from the drawer (Figure 6-4).



**Figure 6-4** Changing battery

- 4 Replace a new 9-volt battery into the drawer, making sure that it is in the correct orientation.

Markings in the drawer will guide you.

- 5 Slide the drawer into the bracket until you feel a click.
- 6 Test the new battery by following the procedure on page 111.

# Periodic Maintenance

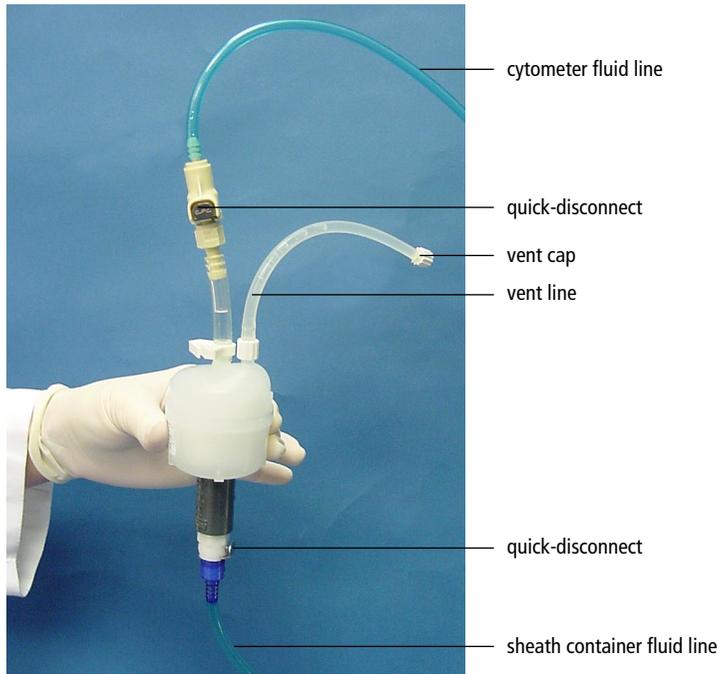
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The following instrument components should be checked occasionally and cleaned as necessary. The frequency will depend on how often the instrument is run. Other components should be checked periodically for wear and replaced if necessary.

- Changing the Sheath Filter on page 114
- Changing the Bal Seal on page 117
- Changing the Sample Tube O-Ring on page 119

## Changing the Sheath Filter

The sheath filter (Figure 6-5 on page 115), connected to the top of the sheath container, filters the sheath fluid as it comes from the sheath container. Increased debris appearing in an FSC vs SSC plot can indicate that the sheath filter needs to be replaced. We recommend changing the sheath filter assembly every 3–6 months.



**Figure 6-5** Sheath filter

- Tip** To avoid spraying sheath fluid, depressurize the sheath container before opening it.

### Remove the Old Filter

- 1** Place the instrument in STNDBY.
- 2** Disconnect the air line (green).
- 3** Depressurize the sheath container by lifting the vent valve.  
For a description of the vent valve, see Figure 2-5 on page 54.
- 4** Detach the cytometer fluid line from the filter assembly by squeezing the quick-disconnect.

- 5** Detach the sheath container fluid line from the filter assembly by squeezing the quick-disconnect.
- 6** Remove the vent line from the filter; set aside.  
Twist to remove.
- 7** Discard the used filter assembly.

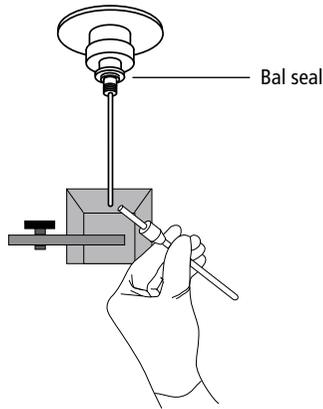
### **Attach the New Filter**

- 1** Connect the vent line to the filter.  
Twist to attach.
- 2** Connect the sheath container fluid line to the filter assembly via the quick-disconnect.
- 3** Attach the cytometer fluid line to the filter assembly via the quick-disconnect.
- 4** Turn on the instrument to pressurize the sheath container.
- 5** Loosen the filter's vent cap to bleed off any air in the sheath filter.
- 6** Carefully tap the filter assembly to dislodge any air trapped in the filter element.
- 7** Loosen the filter's vent cap again to bleed off any air in the sheath filter.

## Changing the Bal Seal

The sample injection tube Bal seal is a Teflon™ ring that forms a seal with the sample tube and ensures proper tube pressurization. Over time, this seal becomes worn or cracked and requires replacement. Replacement is necessary if a proper seal is not formed when a sample tube is installed on the SIP.

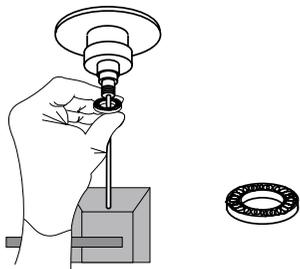
- 1 Remove the outer droplet sleeve from the sample injection tube by turning the retainer counterclockwise.



**Figure 6-6** Removing the outer sleeve

**⚠ NOTE** Work carefully—the outer sleeve can fall out as you loosen the retainer.

- 2 Remove the Bal seal by gripping it between your thumb and index finger and pulling (Figure 6-7).



**Figure 6-7** Removing the Bal seal

- 3 Install the new Bal seal spring-side up.  
Gently push the seal in place to seat it.
- 4 Reinstall the retainer and outer sleeve over the sample injection tube.  
Tighten the retainer just enough to hold it in place.
- 5 Install a sample tube on the SIP to ensure that the outer sleeve has been properly installed.  
If the sleeve hits the bottom of the tube, loosen the retainer slightly and push the sleeve up as far as it will go. Tighten the retainer.

## Changing the Sample Tube O-Ring

The sample tube O-ring, located within the retainer, forms a seal that allows the droplet containment vacuum to function properly. Replace the O-ring when droplets form at the end of the sample injection tube while the vacuum is operating.

 **WARNING** Instrument hardware might be contaminated with biohazardous material. Wear suitable protective clothing, eyewear, and gloves whenever cleaning the instrument or replacing parts.

- 1** Remove the outer droplet sleeve from the sample injection tube.

Turn the retainer counterclockwise and pull the outer sleeve from the retainer (see Figure 6-6 on page 117).

- 2** Invert the retainer and allow the O-ring to fall onto the benchtop.

If the O-ring does not fall out initially, tap the retainer on the benchtop to dislodge the O-ring.

- 3** Drop the new O-ring into the retainer.

Make sure the O-ring is seated properly in the bottom of the retainer.

- 4** Reinstall the retainer and the outer sleeve.

- 5** Install a sample tube on the SIP to ensure that the outer sleeve has been properly installed.

If the sleeve hits the bottom of the tube, loosen the retainer slightly and push the sleeve up as far as it will go. Tighten the retainer.



# 7

## Troubleshooting

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The tips in this section are designed to help you troubleshoot your experiments. Additional troubleshooting information can be found in the *BD FACSDiVa Software User's Guide*.

If additional assistance is required, contact your local BD Biosciences technical support representative. See Technical Assistance on page xiv.

# Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Droplet containment vacuum not functioning	Worn O-ring in retainer	Replace the O-ring. See page 119.
	Outer sleeve not seated in the retainer	<ol style="list-style-type: none"> <li><b>1</b> Loosen the retainer (Figure 6-6 on page 117).</li> <li><b>2</b> Push the outer sleeve up into the retainer until seated.</li> <li><b>3</b> Tighten the retainer.</li> </ol>
	Outer sleeve not on the sample injection tube	<p>Replace the outer sleeve.</p> <ol style="list-style-type: none"> <li><b>1</b> Loosen the retainer.</li> <li><b>2</b> Slide the outer sleeve over the sample injection tube until it is seated.</li> <li><b>3</b> Tighten the retainer.</li> </ol>
	Waste line pinched, preventing proper aspiration	Check the waste line.
	Waste tank full	Empty the waste tank.
Sample tube not fitting on SIP	Sample tube other than BD Falcon™ tubes used	Use BD Falcon 12 x 75-mm sample tubes. See page 160.
	Worn Bal seal	Replace the Bal seal. See page 117.
Rapid sample aspiration	Support arm to the side	Place the support arm under the sample tube.
	Droplet containment module failing	Call your service representative.

# Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in acquisition display and green RUN button	Threshold not set to correct parameter (usually FSC)	Set the threshold to the correct parameter for your application.
	Threshold level too high	Lower the threshold level.
	PMT voltage for threshold parameter set too low	Set the PMT voltage higher for the threshold parameter. Refer to the <i>BD FACSDiVa Software User's Guide</i> for instructions.
	Software issue	Refer to the <i>BD FACSDiVa Software User's Guide</i> for more information on setting gates.
	Air in sheath filter	Purge the filter. See Removing Air Bubbles on page 58.
	No sample in tube	Add sample to tube or install new sample tube.
	Sample not mixed properly	Mix sample to suspend cells.
	Waste tank full	Empty the waste tank.
	PMT voltages set too low or too high for display parameter	Reset PMT voltages.
	Too few events displayed	Increase the number of events to display.

## Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in acquisition display and green RUN button (continued)	Sample injection tube clogged	Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes.  If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 106.
	Bal seal worn	Replace the Bal seal. See page 117.
	Laser warming up	Wait the recommended amount of time for the laser to warm up. <ul style="list-style-type: none"> <li>• 30 min for the 488-nm (blue)</li> <li>• 60 min for the 325-nm (UV)</li> <li>• 30 min for the 355-nm (UV)</li> <li>• 15 min for the 405-nm (violet)</li> <li>• 20 min for the 633-nm (red)</li> </ul>
	Laser not functioning	Verify malfunction by changing the threshold to an alternative laser while running appropriate QC particles. If not successful, contact BD Biosciences.

## Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in acquisition display and orange RUN button	RUN not activated	Press the RUN button.
	Sample tube not installed or not properly seated	Install the sample tube correctly on the SIP.
	Sample tube cracked	Replace the sample tube.
	Sheath container not pressurized	<ul style="list-style-type: none"> <li>• Ensure that the sheath container lid and all connectors are securely seated.</li> <li>• Inspect the O-ring and replace if necessary. See Changing the Sample Tube O-Ring on page 119.</li> </ul>
	Bal seal worn	Replace the Bal seal. See page 117.
	Air leak at sheath container	Ensure that the sheath container lid and all connectors are securely seated.
	Sheath container empty	Fill the sheath container.
Air in sheath filter	Purge the filter. See Removing Air Bubbles on page 58.	

# Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No fluorescent signal	Incorrect fluorochrome assignment	Make sure the Instrument Configuration in the software matches the optical filters in the instrument.
	Wrong filter installed	Make sure the appropriate filter is installed for each fluorochrome. See Changing Optical Filters on page 45.
	Laser not functioning	Verify laser malfunction by changing the threshold to an alternative laser while running appropriate QC particles. If not successful, contact BD Biosciences.
High event rate	Air bubble in sheath filter or flow cell	Remove the air bubble. See page 58.
	Threshold level too low	Increase the threshold level. Refer to the <i>BD FACSDiVa Software User's Guide</i> for instructions.
	PMT voltage for threshold parameter set too high	Set the PMT voltage lower for the threshold parameter. Refer to the <i>BD FACSDiVa Software User's Guide</i> for instructions.
	Sample too concentrated	Dilute the sample.
	Sample flow rate set on HI	Set the sample flow rate to MED or LO.

# Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Low event rate	Threshold level too high	Lower the threshold level. Refer to the <i>BD FACSDiVa Software User's Guide</i> for instructions.
	PMT voltage for threshold parameter set too low	Set the PMT voltage higher for the threshold parameter. Refer to the <i>BD FACSDiVa Software User's Guide</i> for instructions.
	Sample not adequately mixed	Mix the sample to suspend cells.
	Sample too dilute	Concentrate the sample. If the flow rate setting is not critical to the application, set the flow rate switch to MED or HI.
	Sample injection tube clogged	Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes.  If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 106.

## Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Erratic event rate	Sample tube cracked	Replace the sample tube.
	Bal seal worn	Replace the Bal seal. See page 117.
	Sample injection tube clogged	Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes.  If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 106.
	Contaminated sample	Prepare the specimen again, making sure that the tube is clean.
	Worn sheath filter	Replace the filter. See Changing the Sheath Filter on page 114.
Distorted scatter parameters	Instrument settings maladjusted	Optimize the scatter parameters. Refer to the <i>BD FACSDiVa Software User's Guide</i> for instructions.
	Air bubble in sheath filter or flow cell	Purge the air from the filter. See Removing Air Bubbles on page 58.
	Flow cell dirty	Perform the system flush procedure. See page 109.
	Air leak at sheath container	Ensure that the sheath container lid is tight and all connectors are secure.
	Hypertonic buffers or fixative	Replace the buffers and fixative.

## Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Excessive amount of debris in display	Threshold level too low	Increase the threshold level.
	Sheath filter dirty	Replace the filter. See Changing the Sheath Filter on page 114.
	Flow cell dirty	Flush the system. See System Flush on page 109.
	Sample contains excessive amount of debris	Examine the sample under a microscope.
	Stock sheath fluid contaminated	Rinse the sheath container with DI water; then fill with sheath fluid from another (or new lot) bulk container.
High CV	Air bubble in sheath filter or flow cell	Purge the filter. See Removing Air Bubbles on page 58.
	Sample flow rate set too high	Set the sample flow rate lower.
	Air leak at sheath container	Ensure that the sheath container lid is tight and all connectors are secure.
	Flow cell dirty	Flush the system. See System Flush on page 109.
	Poor sample preparation	Repeat sample preparation.
	Sample not diluted in same fluid as sheath fluid	Dilute the sample in the same fluid as you are using for sheath.
	Old or contaminated quality control (QC) particles	Make new QC samples and perform the quality control procedure again.

## Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Poor QC results	Air bubble or debris in flow cell	Prime the fluidics system. See Priming the Fluidics (If Necessary) on page 59.
	Old or contaminated QC particles	Make new QC samples and perform the quality control procedure again.
	Sample not diluted in same fluid as sheath fluid	Dilute the sample in the same fluid as you are using for sheath. If you are running BD CaliBRITE™ beads, dilute them in BD FACSTflow solution and use BD FACSTflow solution for sheath fluid.
	Laser not warmed up	Wait the recommended amount of time for the laser to warm up. <ul style="list-style-type: none"> <li>• 30 min for the 488-nm (blue)</li> <li>• 60 min for the 325-nm (UV)</li> <li>• 30 min for the 355-nm (UV)</li> <li>• 15 min for the 405-nm (violet)</li> <li>• 20 min for the 633-nm (red)</li> </ul>
	Laser not functioning	Contact BD Biosciences.
	Optical alignment problem	Contact BD Biosciences.

# Appendix A

## Technical Overview

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- Fluidics on page 132
- Optics on page 133
- Electronics on page 141

# Fluidics

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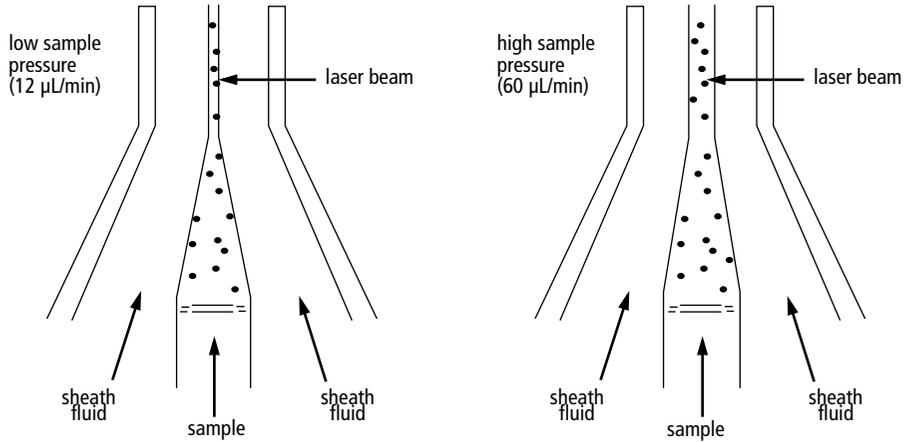
The fluidics system in the BD LSR II flow cytometer is pressure driven—a built-in air pump provides a sheath pressure of 5.5 psi. After passing through the sheath filter, sheath fluid is introduced into the lower chamber of the quartz flow cell.

The sample to be analyzed arrives in a separate pressurized stream. When a sample tube is placed on the sample injection port (SIP), the sample is forced up and injected into the lower chamber of the flow cell by a slight overpressure relative to the sheath fluid. The conical shape of the lower chamber creates a laminar sheath flow that carries the sample core upward through the center of the flow cell, where the particles to be measured are intercepted by the laser beam (Figure A-1 on page 133). This process is known as hydrodynamic focusing.

The objective in flow cytometric analysis is to have one cell or particle moving through the laser beam at a given moment. The difference in pressure between the sample stream and sheath fluid stream can be used to vary the diameter of the sample core. Increasing the sample pressure increases the core diameter and therefore the flow rate (Figure A-1 on page 133).

- A higher flow rate is generally used for qualitative measurements such as immunophenotyping. The data is less resolved but is acquired more quickly.
- A lower flow rate is generally used in applications where greater resolution is critical, such as DNA analysis.

Proper operation of fluidic components is critical for particles to intercept the laser beam properly. Always ensure that the fluidics system is free of air bubbles and debris and is properly pressurized.



**Figure A-1** Hydrodynamic focusing of the sample core through the flow cell

## Optics

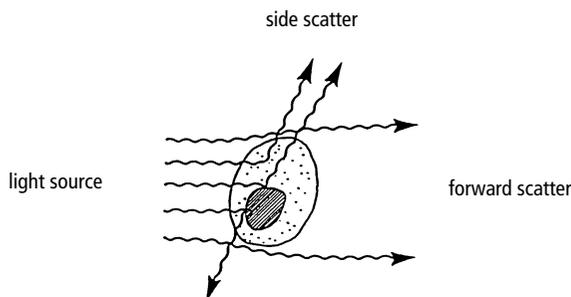
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The optics system consists of lasers, optical filters, and detectors. Lasers illuminate the cells or particles in the sample and optical filters direct the resulting light scatter and fluorescence signals to the appropriate detectors.

### Light Scatter

When a cell or particle passes through a focused laser beam, laser light is scattered in all directions (Figure A-2 on page 134). Light that scatters axial to the laser beam is called forward scatter (FSC); light that scatters perpendicular to the laser beam is called side scatter (SSC). FSC and SSC are related to certain physical properties of cells:

- FSC—indicates relative differences in the size of the cells or particles
- SSC—indicates relative differences in the internal complexity or granularity of the cells or particles



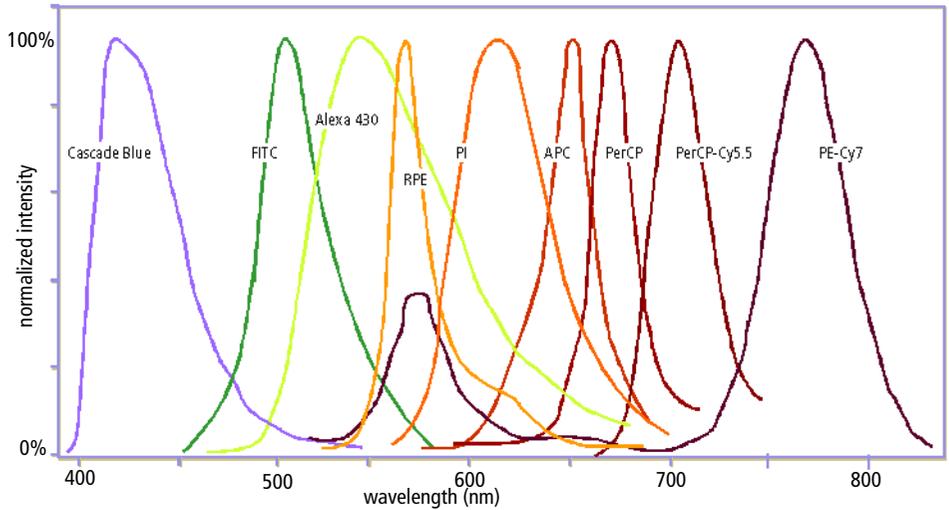
**Figure A-2** Forward scatter (FSC) and side scatter (SSC)

## Fluorescence

When cells or particles stained with fluorochrome-conjugated antibodies or other dyes pass through a laser beam, the dyes can absorb photons (energy) and be promoted to an excited electronic state. In returning to their ground state, the dyes release energy, most of which is emitted as light. This light emission is known as fluorescence.

Fluorescence is always a longer wavelength (lower-energy photon) than the excitation wavelength. The difference between the excitation wavelength and the emission wavelength is known as the Stokes shift. Some fluorescent compounds such as PerCP exhibit a large Stokes shift, absorbing blue light (488 nm) and emitting red light (675 nm), while other fluorochromes such as FITC have a smaller Stokes shift, absorbing blue light and emitting green light (530 nm).

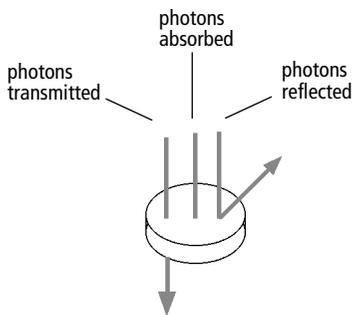
The emission spectra for some commonly used fluorochromes are shown in Figure A-3 on page 135.



**Figure A-3** Emission spectra of commonly used fluorochromes

## Optical Filters

Optical filters modify the spectral distribution of light scatter and fluorescence directed to the detectors. When photons encounter an optical filter, they are either transmitted, absorbed, or reflected (Figure A-4).



**Figure A-4** Effect of an optical filter on incident photons

Even though an optical filter is rated at its 50% transmission point, the filter passes—or lets through—minimal outside of this indicated rating.

The slope of an optical filter transmission curve indicates filter performance. A relatively steep slope indicates a high-performance, high-quality optical filter that provides deep attenuation of out-of-band wavelengths. A less steep slope indicates that more light outside the rated bandwidth is being transmitted.

Two kinds of filters are used on the BD LSR II flow cytometer (default configuration):

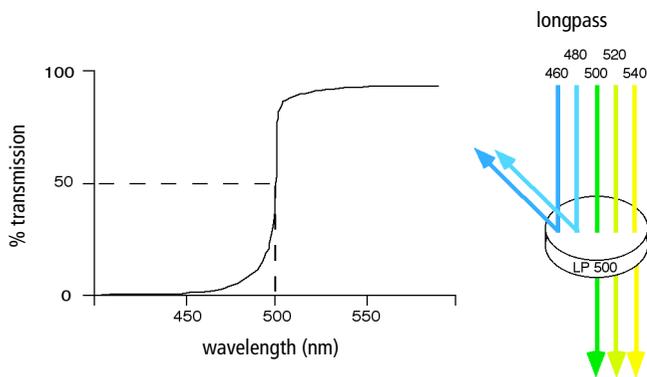
- longpass (LP)
- bandpass (BP), including discriminating filters (DF) and ALPHA filters (AF)

A third filter type, the shortpass (SP), can be used in some of the custom configurations.

LP, BP, and SP filters are referred to as dichroic filters.

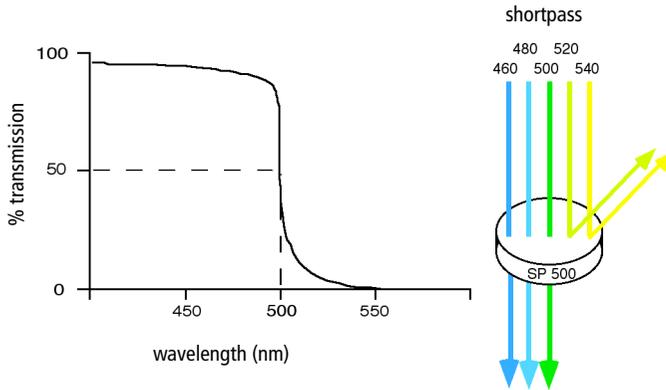
## Longpass Filter

LP filters pass wavelengths longer than the filter rating. For example, a 500-LP filter permits wavelengths longer than 500 nm to pass through it and either absorbs or reflects wavelengths shorter than 500 nm.



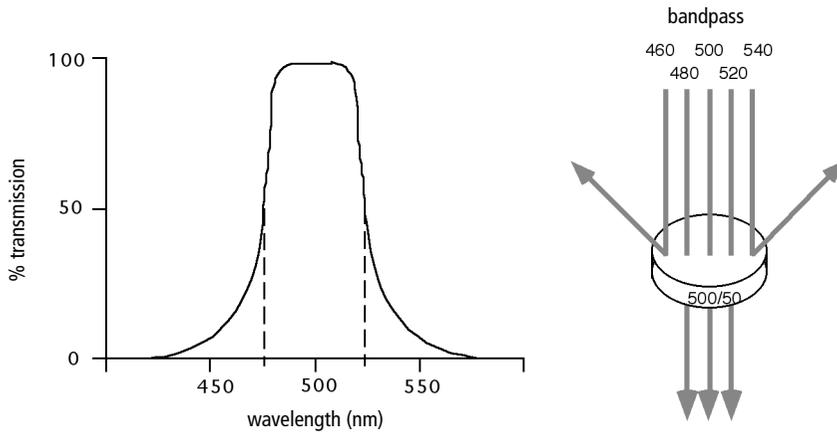
## Shortpass Filter

An SP filter has the opposite properties of a longpass filter. An SP filter passes light with a shorter wavelength than the filter rating.



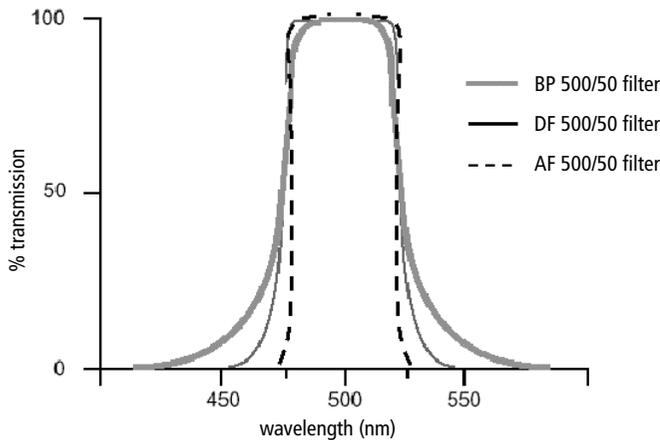
## Bandpass Filter

A BP filter transmits a relatively narrow range or band of light. Bandpass filters are typically designated by two numbers. The first number indicates the center wavelength and the second refers to the width of the band of light that is passed. For example, a 500/50 BP filter transmits light that is centered at 500 nm and has a total bandwidth of 50 nm. Therefore, this filter transmits light between 475 and 525 nm (Figure A-5 on page 138).



**Figure A-5** Bandpass filter

BP and DF filters have the same general function—they transmit a relatively narrow band of light. The principal difference between them is their construction. DF filters have more cavities or layers of optical coatings, resulting in a steeper transmission curve than the curve for a BP filter. This steep slope means that a DF filter is better at blocking light outside the rated bandwidth of the filter.



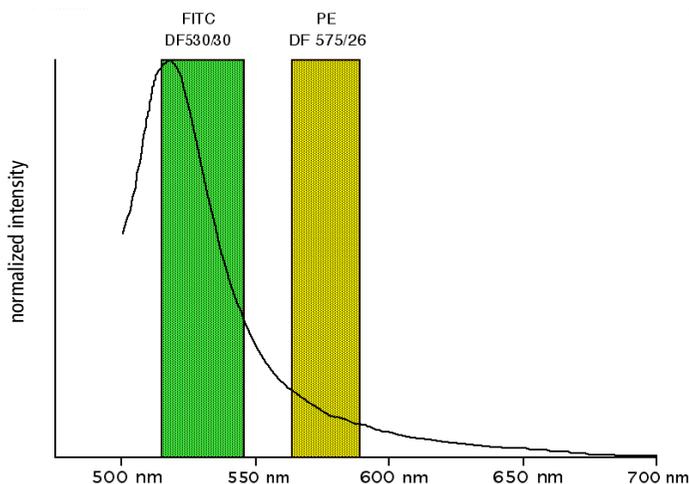
## Dichroic Mirrors

Dichroic filters that are used to direct different color light signals to different detectors are called dichroic mirrors or beam splitters.

Although dichroic mirrors have the properties of LP or SP optical filters, you can not necessarily use any type of LP or SP filter as a beam splitter. A beam splitter must have a surface coating that reflects certain wavelengths, but many types of LP or SP filters are absorbance filters that do not have any specific reflective characteristics. Also, optical filters and beam splitters are rated at a specific angle of incidence. When used in front of the fluorescence detectors, they are perpendicular to the incident light, and when used as a beam splitter, they are placed at an angle relative to the light source. Their optical properties are therefore designed for that angle of incidence.

## Compensation Theory

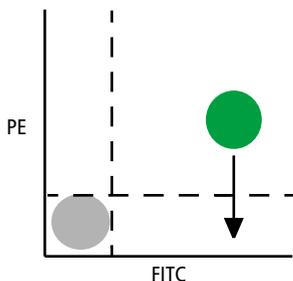
Fluorochromes emit light over a range of wavelengths. As a result, a signal from one fluorochrome can appear in a detector used for another fluorochrome (Figure A-6).



**Figure A-6** Spectral overlap between fluorochromes

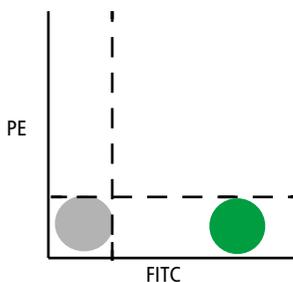
For example, FITC appears primarily in the FITC detector, but some of its fluorescence overlaps into the PE detector. PE appears primarily in the PE detector, but some of its fluorescence overlaps into the FITC detector. This spectral overlap must be corrected or compensated for: hence the term fluorescence compensation.

In Figure A-6, the FITC emission will be detected by both the FITC and PE channels. This can be seen in a dot plot of FITC vs PE (Figure A-7).



**Figure A-7** Theoretical display of FITC vs PE before compensation

This FITC signal must be removed from the PE channel, as indicated by the arrow. Both PE and FITC fluoresce in the yellow (575 nm) range, so there is no way to isolate the emission from each fluorochrome optically. Instead, fluorescence compensation circuitry electronically moves the FITC population down, out of the PE positive area (Figure A-8).



**Figure A-8** FITC signal properly compensated out of the PE channel

Compensation networks are adjusted in the Compensation tab in the Inspector in BD FACSDiVa software. Each compensation network represents a detector from which another fluorochrome's emission is removed. For example, if FITC is detected in the PE channel, the FITC signal must be removed and subtracted from the PE channel. The PE-%FITC compensation circuit in the Compensation tab is therefore used to subtract the FITC signal from the PE channel.

Once fluorescence compensation has been set for one sample, the compensation value remains valid whether a dim or bright sample is run. Figure A-9 demonstrates this principle. Although the signals differ in intensity, the percentage of signal detected in the FITC and PE channels remains constant.

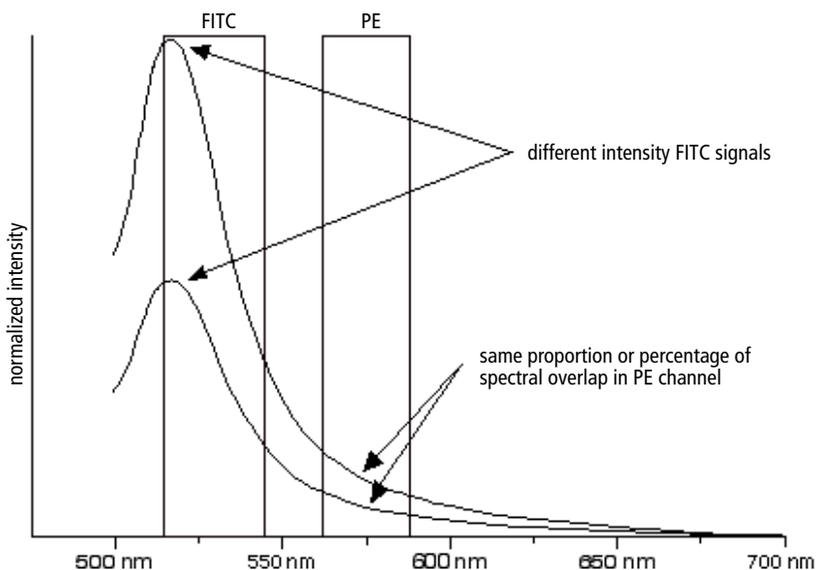


Figure A-9 Two FITC signals of different intensity

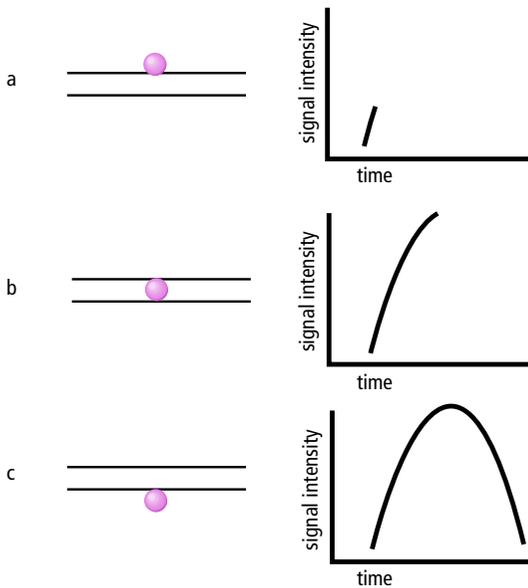
## Electronics

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As cells or other particles pass through a focused laser beam, they scatter the laser light and can emit fluorescence. Because the laser beam is focused on a small spot and particles move rapidly through the flow cell, the scatter or fluorescence

emission has a very brief duration—only a few microseconds. This brief flash of light is converted into an electrical signal by the detectors. The electrical signal is called a pulse (Figure A-10).

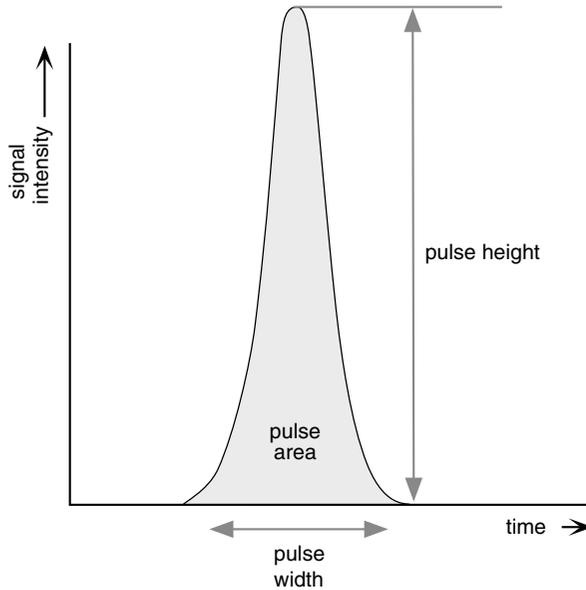
- a** A pulse begins when a particle enters the laser beam. At this point, both the beam intensity and signal intensity are low.
- b** The pulse reaches a maximum intensity or height when the particle reaches the middle of the beam, where the beam and signal intensity are the brightest. The peak intensity, or height of the pulse, is measured at this point.
- c** As the particle leaves the beam, the pulse trails off below the threshold.



**Figure A-10** Anatomy of a pulse

# Pulse Measurements

The pulse processors measure pulses by three characteristics: height, area, and width (Figure A-11).



**Figure A-11** Pulse measurements

The pulse height measures the maximum digitized value for the pulse; the pulse area calculates the sum of all height areas for the pulse; and the pulse width calculates  $\frac{\text{area}}{\text{height}} \times 64000$ .

# Digital Electronics

The electronics found in the BD LSR II flow cytometer digitizes the amount of light signals created by particles passing through the laser beams. The voltages corresponding to these signals are digitized during operation and are represented as numbers in the computer's memory. The instrument hardware calculates

- pulse area and width
- compensation
- ratios

The results are then transferred to your computer for further processing by BD FACSDiVa software.

For more information about digital theory, refer to Appendix B in the *BD FACSDiVa Software User's Guide*. For an in depth discussion, visit our website at [http://www.bdbiosciences.com/immunocytometry\\_systems/](http://www.bdbiosciences.com/immunocytometry_systems/) and download the *BD FACSDiVa Option White Paper*. From the main menu on the Immunocytometry Systems home page, choose Products > Literature > Technical/White Papers. Then choose the *BD FACSDiVa Option White Paper* from the list.

The screenshot shows the BD Biosciences website header and a navigation menu. The BD Biosciences logo is on the left, with the text 'BD Biosciences', 'Clontech', 'Discovery Labware', 'Immunocytometry Systems', and 'Pharmingen' below it. The navigation bar includes 'Home', 'About', 'Products', 'Support', 'Careers', and 'Home'. Below the navigation bar, there is a breadcrumb trail: 'BD Biosciences > Immunocytometry Systems'. The main content area is titled 'IMMUNOCYTOTOMETRY SYSTEMS' and features a large image of a flow cytometer. To the right of the image is a vertical menu with the following items: 'Online Catalog', 'Literature', 'Clontech', 'Discovery Labware', 'Immunocytometry Systems', 'Pharmingen', 'Immune Function', 'Spotlights', 'Brochures', 'Manuals', 'New Product Announcements', 'Newsletters', 'Posters, etc.', 'Sales Sheets', 'Technical/White Papers', and 'Literature Request'. Each item in the menu has a right-pointing arrow next to it.

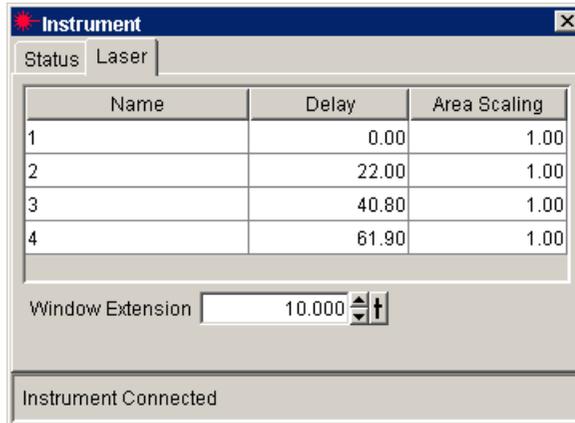
# Threshold

The threshold defines the level at which the system starts to measure the signal pulses. The system monitors the data and calculates pulse area and height for all channels each time a signal exceeds the threshold.

# Laser Controls

Controls in the Laser tab of the Instrument frame are used to set the laser delay to capture signals from multiple lasers. These parameters are set by BD Biosciences service personnel when the BD LSR II flow cytometer is installed and rarely need to be changed.

Record and save Delay values, Area Scalings values, and Window Extension settings for future reference.



**NOTE** Do not change the settings in the Laser tab unless instructed to do so by BD Biosciences. Changing the settings will affect your data.



# Appendix B

## BD LSR II QC Log

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This sample quality control (QC) log can be photocopied or used as a guide in designing your own QC log.



Instrument Serial Number/Name		Calibration Particle				Lot #
Date						
FSC	Mean					
	CV					
SSC	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
Operator Initials						



# Appendix C

## Filter Templates

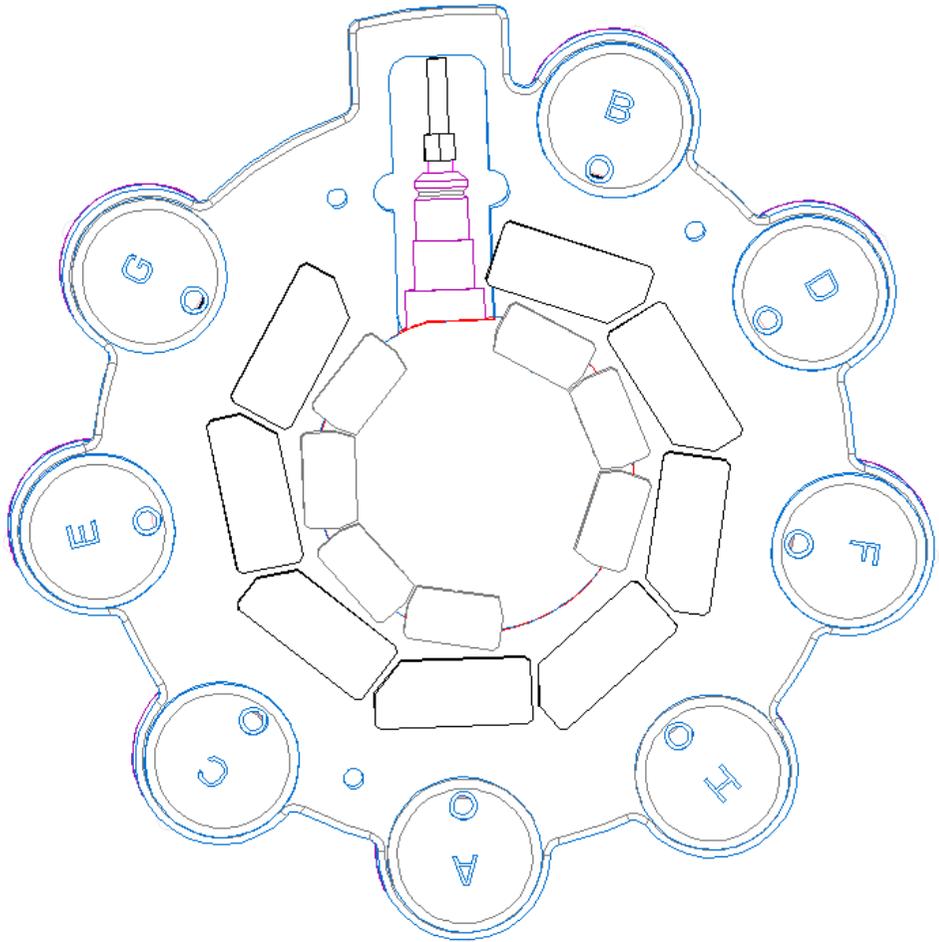
---

You can use these templates to note your custom filter configurations for the BD LSR II instrument.



# Octagon Template

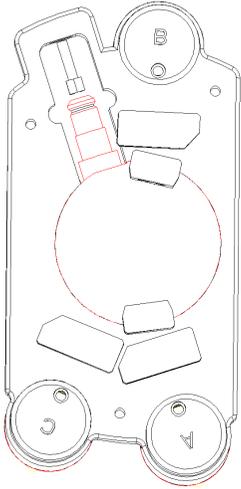
Experiment \_\_\_\_\_



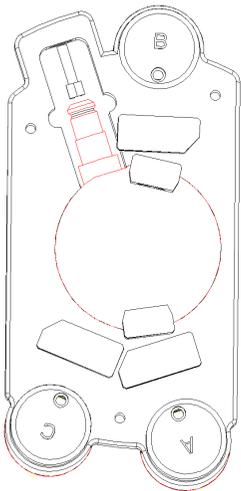


# Trigon Templates

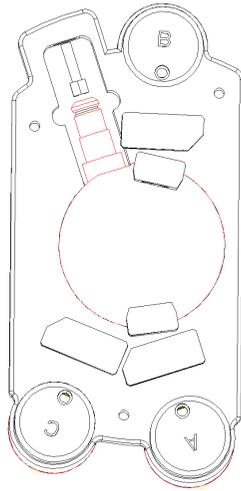
Experiment \_\_\_\_\_



Experiment \_\_\_\_\_



Experiment \_\_\_\_\_





# Appendix D

## Supplies and Consumables

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- Contact Information on page 158
- Consumables on page 158

## Contact Information

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To order spare parts and consumables, such as bulk fluids, from BD Biosciences within the US, call: (877) 237-8995, prompt #2-2-4.

For other countries, contact your local BD Biosciences customer service representative. Worldwide contact information can be found at [www.bdbiosciences.com](http://www.bdbiosciences.com).

## Consumables

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### QC Particles

Particle	Laser	Supplier	Catalog No.
<ul style="list-style-type: none"><li>• BD CaliBRITE 3 beads (unlabeled, FITC, PE, PerCP)</li><li>• BD CaliBRITE APC beads</li></ul>	<ul style="list-style-type: none"><li>• blue 488-nm</li><li>• red 633-nm</li></ul>	BD Biosciences Immunocytometry Systems	<ul style="list-style-type: none"><li>• 340486</li><li>• 340487</li></ul>
<ul style="list-style-type: none"><li>• SPHERO™ Rainbow Calibration Particles (8 peak)</li><li>• SPHERO Ultra Rainbow Fluorescent Particles (single peak)</li></ul>	<ul style="list-style-type: none"><li>• all</li><li>• all</li></ul>	<ul style="list-style-type: none"><li>• BD Biosciences Pharmingen</li><li>• Spherotech, Inc.</li></ul>	<ul style="list-style-type: none"><li>• 559123</li><li>• URFP-30-2</li></ul>
DNA QC Particles kit	blue 488 nm	BD Biosciences Immunocytometry Systems	349523

## Reagents

Reagent	Supplier	Catalog No.
BD FACSThrow sheath fluid	BD Biosciences	340398 (US and Latin America) 342003 (Europe)
Monoclonal antibodies	BD Biosciences	– <sup>a</sup>
BD FACSThrow Lysing Solution	BD Biosciences	349202
BD FACSThrow Rinse detergent	BD Biosciences	340346
BD FACSThrow Clean solution	BD Biosciences	340345
Dyes and fluorochromes	BD Biosciences • Clontech • Pharmingen Molecular Probes Sigma	–
Chlorine bleach (5% sodium hypochlorite)	Clorox or other major supplier (to ensure that the bleach is at the correct concentration and free of particulate matter)	–

a. Refer to the BD Biosciences Immunocytometry Systems Products Catalog or the BD Biosciences website (<http://www.bdbiosciences.com>).

# Equipment

Equipment Item	Supplier	Catalog No.
Bal seal	BD Biosciences	343509
O-ring, sample tube		343615
Sheath filter assembly		344678
BD Falcon polystyrene test tubes, 12 x 75-mm	BD Labware	352052 352054 352058

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